ROBERTA RÚBIA PINTO NOGUEIRA LIMA

STUDY OF SIDA YELLOW SPOT VIRUS, A BEGOMOVIRUS WITH A DIVERGENT COAT PROTEIN

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Doctor Scientiae*.

Orientador: Francisco Murilo Zerbini

VIÇOSA - MINAS GERAIS
2022

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
L732s
2022

Lima, Roberta Rúbia Pinto Nogueira, 1992-
Study of Sida yellow spot virus, a begomovirus with a divergent coat protein / Roberta Rúbia Pinto Nogueira Lima. – Viçosa, MG, 2022.
1 tese eletrônica (89 f.): il. (algumas color.).

Texto em inglês.
Orientador: Francisco Murilo Zerbini Júnior.
Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Fitopatologia, 2022.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2023.145
Modo de acesso: World Wide Web.

1. Begomovírus. 2. Geminivírus. 3. Proteínas. 4. Vírus de plantas. I. Zerbini Júnior, Francisco Murilo, 1966-.
II. Universidade Federal de Viçosa. Departamento de Fitopatologia. Programa de Pós-Graduação em Fitopatologia.
III. Título.

CDD 22. ed. 579.28

Bibliotecário(a) responsável: Bruna Silva CRB-6/2552

ROBERTA RÚBIA PINTO NOGUEIRA LIMA

STUDY OF SIDA YELLOW SPOT VIRUS, A BEGOMOVIRUS WITH A DIVERGENT COAT PROTEIN

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Doctor Scientiae*.

APROVADA: 23 de dezembro de 2022.

Assentimento:

Documento assinado digitalmente
gov.br
ROBERTA RUBIA PINTO NOGUEIRA LIMA
Data: 20/04/2023 12:05:22-0300
Verifique em https://validar.iti.gov.br

Roberta Rúbia Pinto Nogueira Lima
Autora

Documento assinado digitalmente
gov.br
FRANCISCO MURILO ZERBINI JUNIOR
Data: 20/04/2023 13:19:12-0300
Verifique em https://validar.iti.gov.br

Francisco Murilo Zerbini Júnior
Orientador

AGRADECIMENTOS

Aos meus pais, Roberto e Vilma, pelo amor, dedicação, apoio incondicional, confiança e pela educação em todos os sentidos da palavra.

À minha irmã, Vanessa, pelo carinho, amizade, incentivo, distrações e por ser meu porto seguro em qualquer momento da vida.

Ao Perry, por todo o suporte emocional, pelo amor simples e incondicional e por todos os abraços felpudos.

Ao Lucas, por segurar minha mão com tanto carinho e caminhar comigo até o final, por fazer com que fosse possível finalizar esse trabalho.

Às minhas eternas e melhores amigas, Vanessa e Luíza, por estarem comigo em todos os bons e maus momentos, por todas as risadas, campeonatos e Roselis.

Ao handebol feminino da LUVE e da Engenharia pela confiança, pelas alegrias das vitórias e ensinamentos das derrotas, pelo trabalho em grupo e por serem a minha maior válvula de escape e meu maior prazer.

À minha família pelo carinho e pela paciência em todos os momentos ausentes.

Ao professor Murilo Zerbini pelo acolhimento, pela confiança, pela orientação, pelo aprendizado e por acreditar até o fim.

Aos meus colegas do Laboratório de Ecologia e Evolução de Vírus, Lucas, Ana Reis, Ruither, Narla, Anelise, Baltazar e Patrícia por toda a ajuda e companheirismo. Em especial ao João Paulo pelos anos de amizade e todos os bons momentos dentro e fora do laboratório.

A todos os professores que foram fundamentais para a minha formação desde os meus primeiros passos.

Ao departamento de Fitopatologia pela oportunidade e por toda a infraestrutura.

Ao Núcleo de Microscopia e Microanálise da UFV e ao Centro de Microscopia da UFMG.

Ao professor Jônatas Abrahão e à doutoranda Isabella Aquino, pela disponibilidade em intermediar o contato com o CM.

À UFV por ter sido minha casa durante os 12 anos que vivi aqui, pela sua excelência e por ser a mais linda do Brasil.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001, a quem também agradeço.

RESUMO

LIMA, Roberta Rúbia Pinto Nogueira, D.Sc., Universidade Federal de Viçosa, dezembro de 2022. **Study of sida yellow spot virus, a begomovirus with a divergent coat protein.** Orientador: Francisco Murilo Zerbini Júnior.

Vírus do gênero *Begomovirus* (família *Geminiviridae*) são considerados um dos patógenos de plantas mais relevantes devido às severas perdas causadas em cultivos economicamente importantes. Begomovirus são transmitidos pela mosca branca *Bemisia tabaci* de forma persistente-circulativa, e a única proteína viral necessária para aquisição e transmissão pelo inseto vetor é proteína capsidial (CP). A CP é responsável pela especificidade pelo vetor e controla o transporte das partículas virais através do intestino da mosca branca, protegendo o vírus de degradação por enzimas, através de sua interação com a proteína GroEL produzida por bactérias endossimbióticas. Embora a CP seja a proteína mais conservada dos begomovírus, recentemente foi descrito um begomovírus bipartido com uma CP altamente divergente, denominado Sida yellow spot virus (SiYSV), infectando a planta não-cultivada *Sida acuta* (*Malvaceae*). Os objetivos desse trabalho foram determinar se o SiYSV é capaz de formar partículas geminadas, e realizar a caracterização biológica e molecular do SiYSV. Para o primeiro objetivo, a estrutura 3D da CP do SiYSV foi predita usando o AlphaFold e comparada, pelo mTM-align, com outras CP´s típicas de begomovírus. Além disso, cortes ultrafinos de plantas infectadas foram observados ao microscópio eletrônico de transmissão para detecção de partículas virais. Embora muito diferentes em sequência, a CP do SiYSV assume uma conformação 3D similar a de outros begomovírus. No entanto, partículas não foram observadas ao MET. Para o segundo objetivo, a gama de hospedeiros do SiYSV foi determinada e um ensaio de transmissão por mosca branca foi realizado. *B. tabaci* MEAM1 não transmitiu o SiYSV, e a interação entre a CP e a GroEL não foi observada por BiFC. Além disso, a localização subcelular das proteínas codificadas pelo SiYSV foi observada ao microscópio confocal e sua capacidade de suprimir o silenciamento de RNA foi analisada. Os resultados indicam que essas proteínas tiveram o mesmo padrão de localização esperado para begomovírus típicos, mas não foi observada nenhuma atividade de supressão de silenciamento. Juntos, esses resultados indicam que as proteínas codificadas pelo SiYSV podem atuar como as dos demais begomovírus, portanto a possibilidade de que o SiYSV forme um capsídeo não pode ser descartada mesmo que partículas ainda não tenham sido observadas. Ainda, a incapacidade de

B. tabaci transmitir o SiYSV pode não estar relacionada a sua impossibilidade em formar partículas, mas sim a falta de interação entre a CP e a GroEL.

Palavras-chave: Geminivírus. Proteína capsidial. *Bemisia tabaci.*

ABSTRACT

LIMA, Roberta Rúbia Pinto Nogueira, D.Sc., Universidade Federal de Viçosa, December, 2022. **Study of sida yellow spot virus, a begomovirus with a divergent coat protein.** Advisor: Francisco Murilo Zerbini Júnior.

Viruses of the genus *Begomovirus* (family *Geminiviridae*) are considered one of the most relevant plant pathogens due to the severe losses that they cause in economically important crops. Begomoviruses are transmitted by the whitefly *Bemisia tabaci* in a persistent-circulative manner, and the only viral protein required for acquisition and transmission by the insect vector is the capsid protein (CP). The CP mediates vector specificity and controls virus transport through the whitefly gut, protecting the virus from degradation, through interaction with a GroEL homologue produced by endosymbiotic bacteria. Although the CP is the most conserved begomovirus protein, we have described a bipartite begomovirus with a highly divergent CP, named Sida yellow spot virus (SiYSV), infecting the non-cultivated plant *Sida acuta* (Malvaceae). The objectives of this work were to determine whether SiYSV is capable of forming geminate particles, and to carry out the biological and molecular characterization of SiYSV. For the first objective, the 3D structure of the SiYSV CP was predicted using AlphaFold and compared with other typical begomovirus CP's by mTM-align. Then, ultrathin sections of infected plants were observed under a transmission electron microscope for detection of viral particles. Although very different in sequence, the SiYSV CP assumes a 3D conformation similar to that of other begomoviruses. However, particles were not observed under the TEM. For the second objective, the SiYSV host range was assessed, and a whitefly transmission assay was performed. *B. tabaci* MEAM1 was not able to transmit SiYSV, and an interaction between the CP and GroEL was not observed by BiFC. Furthermore, the subcellular localization of SiYSV-encoded proteins was observed on a confocal microscope and their ability to suppress RNA silencing was analyzed. The results indicate that these proteins had the same pattern of localization expected for typical begomoviruses, but we could not observe any strong silencing suppression activity. Together, these results indicate that the SiYSV-encoded proteins can function just as those of other begomoviruses, thus the possibility that SiYSV forms its capsid cannot be discarded even though we have not yet observed its particles. Also, the inability of *B. tabaci* to transmit SiYSV may not be related to the inability to form viral particles, but rather to the lack of interaction between CP and GroEL.

Keywords: Geminivirus. Coat protein. *Bemisia tabaci*.

SUMMARY

GENERAL INTRODUCTION

Viruses are considered one of the most devastating plant pathogens, second only to fungi in the number of crop diseases and their socioeconomic impacts (Nicaise, 2014). It has been reported that viruses can cause losses in crop yields above 60% and, unfortunately, control measures are less effective than for other pathogens (Nicaise, 2014). Furthermore, viruses have been considered "the major class of emerging pathogens" (García-Arenal and Zerbini, 2019) as a great number of diseases that has emerged in the last decades are caused by viruses (Anderson et al., 2004; García-Arenal and Zerbini, 2019; Taylor et al., 2001). Even more worrying is that emergence is usually associated with increased virulence (Anderson et al., 2004; Fraile and Garcia-Arenal, 2016; García-Arenal and Zerbini, 2019) causing several losses, as witnessed recently with the outbreak of a coronavirus (SARS-CoV-2) infecting humans.

Among the plant viruses, those classified in the family *Geminiviridae* are among the most relevant as they can cause severe losses in economically important crops (Rojas et al., 2018). Geminiviruses can infect tomato, cotton, cassava, cucurbits and beans, mostly in tropical and subtropical areas (Legg and Fauquet, 2004; Moriones and Navas-Castillo, 2000; Navas-Castillo et al., 2011; Rojas et al., 2018), although their prevalence in temperate regions has been increasing (García-Arenal and Zerbini, 2019).

The family *Geminiviridae* is comprised of viruses with one or two genomic components of circular, single-stranded DNA (ssDNA) of 2,600-2,800 nucleotides (nt) in length, encapsidated in geminate, quasi-icosahedral particles and transmitted by different insect vectors (Fiallo-Olive et al., 2021). These viruses replicate their genomes by rolling-circle and recombination-dependent mechanisms (Jeske et al., 2001; Stenger et al., 1991), and they all have an intergenic region (IR, also known as common region, CR, in viruses with bipartite genomes) containing highly conserved elements. The IR includes a stem-loop structure with a

sequence of nine nucleotides (5'-TAATATTAC-3') that constitutes the origin of replication, and also contains direct and inverted repeated sequences of four or five nucleotides, called iterons. These iterons are essential for origin recognition by the viral protein associated to the replication process (Rojas et al., 2005). According to their genomic organization, host range, insect vector and phylogenetic relationships, the members of the *Geminiviridae* family are divided into 14 genera: *Becurtovirus*, *Begomovirus*, *Capulavirus*, *Citlodavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Maldovirus*, *Mastrevirus*, *Mulcrilevirus*, *Opunvirus*, *Topilevirus*, *Topocuvirus* and *Turncurtovirus* (Fiallo-Olive et al., 2021).

The genus *Begomovirus* constitutes the group with the highest number of species and is the most economically important. According to their genome architecture and phylogenetic relationships, begomoviruses are divided into two major groups: New World (NW - the Americas) and Old World (OW - Africa, Asia and Europe) (Briddon et al., 2010). Begomoviruses from the NW are predominantly bipartite and their genome components are named DNA-A and DNA-B. The DNA-A encodes proteins related to replication, encapsidation and suppression of host defenses (Hanley-Bowdoin et al., 2013; Rojas et al., 2005). In turn, the DNA-B encodes proteins involved in intracellular, cell-to-cell movement and systemic infection, host range determination, and suppression of host defenses (Brustolini et al., 2015; Hanley-Bowdoin et al., 2013; Rojas et al., 2005). The OW begomoviruses can be monopartite or bipartite and are usually associated with satellite DNA molecules (Zhou, 2013). The single genomic component of monopartite begomoviruses is homologous to the DNA-A of bipartite begomoviruses (Hanley-Bowdoin et al., 1999).

There are several additional features that distinguish NW and OW begomoviruses. The most outstanding difference is the presence of an additional open reading frame (ORF) in OW viruses, which partially overlaps the capsid protein (CP) gene. This ORF, named V2/AV2 in monopartite or bipartite begomoviruses, respectively, encodes a protein involved in viral

movement and gene silencing suppression (Glick et al., 2008; Padidam et al., 1996; Rybicki, 1994). Other genomic features that distinguish between these two groups include: (i) OW begomoviruses have ~100 nt longer components (Ho et al., 2014); (ii) NW begomoviruses have a PWRsMaGT motif in the N-terminal region of the CP, absent in OW viruses (Ha et al., 2006; Harrison et al., 2002); (iii) the CP N-terminal region from OW begomoviruses has two or three basic domains, typically KR, KVRRR and K/RRRR, that are part of a nuclear localization signal (NLS), while NW begomoviruses have only the first (KR) domain (Ha et al., 2008); (iv) NW begomoviruses have a tyrosine phosphorylation site with the consensus sequence RKx(2,3)DEx(2,3)Y in the movement protein (MP), which is absent in OW begomoviruses (Ho et al., 2014).

The emergence of begomoviruses as serious pathogens in many economically important crops has been associated with changes in crop cultivation and cropping practices, with the increased movement of plants worldwide, and with the increased prevalence of their insect vectors, whiteflies of the *Bemisia tabaci* complex (Hemiptera: Aleyrodidae) (Navas-Castillo et al., 2011). *Bemisia tabaci* is a cryptic species complex with at least 39 morphologically identical species which can be distinguished by the sequence of the mitochondrial gene cytochrome oxidase I (mtCOI). Two species are the most relevant to agriculture, as they are highly invasive pests: *B. tabaci* Mediterranean (MED) and *B. tabaci* Middle East-Asia Minor 1 (MEAM1), previously known as biotypes Q and B, respectively (Vyskočilová et al., 2018). In particular, the introduction and quick dissemination of *B. tabaci* MEAM1 throughout the world has greatly facilitated the emergence and spread of begomoviruses (Gilbertson et al., 2015; Navas-Castillo et al., 2011).

Begomoviruses are transmitted by whiteflies in a persistent-circulative manner. Once the vector acquires the virus from an infected plant, the viral particles move through the insect body, but do not replicate. The virus passes along the gut lumen until reaching the hemolymph.

From there, it is translocated to the salivary glands and, consequently, to the insect's saliva. Finally, the viral particles are ready to be transmitted to a new plant as the whitefly feeds on it and secrets saliva (Czosnek et al., 2017; Hogenhout et al., 2008).

The only viral protein required for acquisition and transmission by the insect vector is the CP (Harrison et al., 2002). The CP mediates vector specificity and controls virus transport through the whitefly gut until it reaches the hemolymph, which contains proteases and nucleases that can destroy the viral particles. To protect the virus from degradation, the CP interacts with a GroEL homologue produced by endosymbiotic bacteria (Czosnek et al., 2017; Harrison et al., 2002; Hogenhout et al., 2008).

In addition to viral genome packing and virus transmission, the CP seems to be involved in several other functions, such as shuttling of viral DNA in and out of the nucleus, viral DNA accumulation, cell-to-cell movement and systemic spread of the virus (Fondong, 2013; Harrison et al., 2002). The CP is capable of binding ssDNA and then shuttles the viral genome to the nucleus, where the infection begins. The newly synthesized CP has a nuclear localization signal (NLS) that mediates its own trafficking from the cytoplasm to the nucleus, where CP accumulates (Fondong, 2013; Harrison et al., 2002; Kunik et al., 1998; Rojas et al., 2001). It also has a nuclear export signal (NES) that redirects the formed viral particles from the nucleus to the cytoplasm and then to the cell wall, from where they can spread cell-to-cell (Fondong, 2013; Harrison et al., 2002; Ward and Lazarowitz, 1999).

The multifunctional nature of the CP places strong constraints in its capacity to vary without losing fitness. In fact, the CP is the most conserved begomovirus protein (Harrison et al., 2002). Nevertheless, our research group has described a bipartite begomovirus with a highly divergent CP, Sida yellow spot virus (SiYSV) (Xavier, 2015). Phylogenetic analyses based on the DNA-A, DNA-B and the Rep gene clustered this new virus with NW begomoviruses. The DNA-A tree grouped SiYSV with begomoviruses present in Brazil, Central America and the

Caribbean, and the DNA-B analysis grouped it with begomoviruses infecting cultivated and non-cultivated hosts in Brazil (Xavier, 2015).

However, analysis of the full-length genome of SiYSV detected several features of OW begomoviruses: (*i*) both the DNA-A and DNA-B components have lengths in the range of OW begomoviruses; (*ii*) the DNA-A contains an AV2-like gene in the virion-sense strand; (*iii*) the presence of conserved motifs that are characteristic of OW begomovirus proteins, including the KVRRR motif in the N-terminal region of the CP. Despite this, SiYSV has a NW-like iteron organization and a tyrosine phosphorylation site in the movement protein (MP), which is present in NW but not in OW begomoviruses (Xavier, 2015).

Although the genomic organization of SiYSV is typical of begomoviruses, there is a region of 1,100 nt in the DNA-A with very low similarity to other begomoviruses, and this region includes the CP, AV2 and part of the common region. Further analysis found a domain related to geminivirus CPs in the putative CP gene but failed to detect any further similarity with the CP and AV2 proteins of other begomoviruses (Xavier, 2015). The only similarity found was with a recently described (and also highly divergent) monopartite geminivirus infecting apple trees in China, called "apple geminivirus" (AGV) (Liang et al., 2015). Even so the similarity between AGV and SiYSV is very low: the SiYSV CP shares 26% nucleotide (nt) identity (99% coverage, E value $4e^{-9}$) and the AV2-like protein shares 33% nt identity (76% coverage, E value $4e^{-12}$) (Xavier, 2015).

SiYSV was found infecting the non-cultivated plant *Sida acuta* (family Malvaceae) (Xavier, 2015). As mentioned before, a great number of new begomoviruses have emerged in the last decades, many of which have been found in non-cultivated plants. Such non-cultivated plants may be considered as wild reservoirs of viral diversity and of new viruses which could spillover and cause diseases in crops (Castillo-Urquiza et al., 2008; Ferro et al., 2017; Fiallo-Olivé et al., 2012; García-Arenal and Zerbini, 2019; Tavares et al., 2003).

Interest and concerns about virus emergence have driven ecological and evolutionary studies, which include the identification of wild host reservoirs and of the evolutionary processes that guide virus adaptation to a new host (García-Arenal and Zerbini, 2019). Adaptation requires genetic variation, so that natural selection can play its role (García-Arenal and Zerbini, 2019). The major source of genetic variation in geminiviruses is mutation (Harkins et al., 2009; Lima et al., 2017), followed by recombination (Briddon et al., 2010; García-Arenal and Zerbini, 2019; Padidam et al., 1999). Evidence of interspecies recombination involving both the DNA-A and the DNA-B of SiYSV was found. In this particular case, the gene encoding the divergent CP is a clear candidate for having a recombinant origin (Xavier, 2015).

Genetic variation must lead to virus adaptation to a new host and/or to its vector if the emerging virus is to be efficiently transmitted within the new host population (García-Arenal and Zerbini, 2019). It is well established that the CP is essential for vector transmission. However, this leaves us with some intriguing questions: is a virus with a highly divergent CP capable of forming particles and, consequently, of being transmitted? If so, is it able to spillover its non-cultivated host and to adapt to a new, cultivated host?

Thus, the objectives of this work are: (i) to determine whether the divergent begomovirus SiYSV is capable of forming geminate particles; (ii) to carry out the biological and molecular characterization of SiYSV; (iii) to study the ecological and evolutionary dynamics of SiYSV. The indispensable role of the CP in the viral infection cycle makes its study relevant to understand the epidemiological dynamics of begomoviruses, with scientific and economic implications.

REFERENCES

Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R., Daszak, P., 2004. Emerging infectious diseases of plants: Pathogen pollution, climate change and agrotechnology drivers. Trends Ecol Evol 19, 535-544.

Briddon, R.W., Patil, B.L., Bagewadi, B., Nawaz-ul-Rehman, M.S., Fauquet, C.M., 2010. Distinct evolutionary histories of the DNA-A and DNA-B components of bipartite begomoviruses. BMC Evol Biol 10, 97.

Brustolini, O.J., Machado, J.P., Condori-Apfata, J.A., Coco, D., Deguchi, M., Loriato, V.A., Pereira, W.A., Alfenas-Zerbini, P., Zerbini, F.M., Inoue-Nagata, A.K., Santos, A.A., Chory, J., Silva, F.F., Fontes, E.P., 2015. Sustained NIK-mediated antiviral signalling confers broad-spectrum tolerance to begomoviruses in cultivated plants. Plant Biotechnol J 13, 1300-1311.

Castillo-Urquiza, G.P., Beserra Jr., J.E.A., Bruckner, F.P., Lima, A.T.M., Varsani, A., Alfenas-Zerbini, P., Zerbini, F.M., 2008. Six novel begomoviruses infecting tomato and associated weeds in Southeastern Brazil. Arch Virol 153, 1985-1989.

Czosnek, H., Hariton-Shalev, A., Sobol, I., Gorovits, R., Ghanim, M., 2017. The incredible journey of begomoviruses in their whitefly vector. Viruses 9, 273.

Ferro, C.G., Silva, J.P., Xavier, C.A.D., Godinho, M.T., Lima, A.T.M., Mar, T.B., Lau, D., Zerbini, F.M., 2017. The ever increasing diversity of begomoviruses infecting non-cultivated hosts: new species from *Sida* spp. and *Leonurus sibiricus*, plus two New World alphasatellites. Ann Appl Biol 170, 204-218.

Fiallo-Olive, E., Lett, J.M., Martin, D.P., Roumagnac, P., Varsani, A., Zerbini, F.M., Navas-Castillo, J., 2021. ICTV Virus Taxonomy Profile: *Geminiviridae* 2021. J Gen Virol 102, 001696.

Fiallo-Olivé, E., Navas-Castillo, J., Moriones, E., Martinez-Zubiaur, Y., 2012. Begomoviruses infecting weeds in Cuba: Increased host range and a novel virus infecting *Sida rhombifolia*. Arch Virol 157, 141-146.

Fondong, V.N., 2013. Geminivirus protein structure and function. Mol Plant Pathol 14, 635-649.

Fraile, A., Garcia-Arenal, F., 2016. Environment and evolution modulate plant virus pathogenesis. Curr Opin Virol 17, 50-56.

García-Arenal, F., Zerbini, F.M., 2019. Life on the edge: geminiviruses at the interface between crops and wild plant hosts. Annu Rev Virol 6, 411-433.

Gilbertson, R.L., Batuman, O., Webster, C.G., Adkins, S., 2015. Role of the insect supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. Annu Rev Virol 2, 67-93.

Glick, E., Zrachya, A., Levy, Y., Mett, A., Gidoni, D., Belausov, E., Citovsky, V., Gafni, Y., 2008. Interaction with host SGS3 is required for suppression of RNA silencing by tomato yellow leaf curl virus V2 protein. Proc Natl Acad Sci USA 105, 157-161.

Ha, C., Coombs, S., Revill, P., Harding, R., Vu, M., Dale, J., 2006. Corchorus yellow vein virus, a New World geminivirus from the Old World. J Gen Virol 87, 997-1003.

Ha, C., Coombs, S., Revill, P., Harding, R., Vu, M., Dale, J., 2008. Molecular characterization of begomoviruses and DNA satellites from Vietnam: Additional evidence that the New World geminiviruses were present in the Old World prior to continental separation. J Gen Virol 89, 312-326.

Hanley-Bowdoin, L., Bejarano, E.R., Robertson, D., Mansoor, S., 2013. Geminiviruses: Masters at redirecting and reprogramming plant processes. Nat Rev Microbiol 11, 777-788.

Hanley-Bowdoin, L., Settlage, S.B., Orozco, B.M., Nagar, S., Robertson, D., 1999. Geminiviruses: Models for plant DNA replication, transcription, and cell cycle regulation. Crit Rev Plant Sci 18, 71-106.

Harkins, G.W., Delport, W., Duffy, S., Wood, N., Monjane, A.L., Owor, B.E., Donaldson, L., Saumtally, S., Triton, G., Briddon, R.W., Shepherd, D.N., Rybicki, E.P., Martin, D.P., Varsani, A., 2009. Experimental evidence indicating that mastreviruses probably did not co-diverge with their hosts. Virol J 6, 104.

Harrison, B.D., Swanson, M.M., Fargette, D., 2002. Begomovirus coat protein: serology, variation and functions. Physiol Mol Plant Pathol 60, 257-271.

Ho, E.S., Kuchie, J., Duffy, S., 2014. Bioinformatic analysis reveals genome size reduction and the emergence of tyrosine phosphorylation site in the movement protein of New World bipartite begomoviruses. PLOS ONE 9, e111957.

Hogenhout, S.A., Ammar, E.D., Whitfield, A.E., Redinbaugh, M.G., 2008. Insect vector interactions with persistently transmitted viruses. Annu Rev Phytopathol 46, 327-359.

Jeske, H., Lutgemeier, M., Preiss, W., 2001. DNA forms indicate rolling circle and recombination-dependent replication of *Abutilon mosaic virus*. EMBO J 20, 6158-6167.

Kunik, T., Palanichelvam, K., Czosnek, H., Citovsky, V., Gafni, Y., 1998. Nuclear import of the capsid protein of tomato yellow leaf curl virus (TYLCV) in plant and insect cells. Plant J 13, 393-399.

Legg, J., Fauquet, C., 2004. Cassava mosaic geminiviruses in Africa. Plant Mol Biol 56, 585-599.

Liang, P., Navarro, B., Zhang, Z., Wang, H., Lu, M., Xiao, H., Wu, Q., Zhou, X., Di Serio, F., Li, S., 2015. Identification and characterization of a novel geminivirus with monopartite genome infecting apple trees. J Gen Virol 96, 2411-2420.

Lima, A.T.M., Silva, J.C.F., Silva, F.N., Castillo-Urquiza, G.P., Silva, F.F., Seah, Y.M., Mizubuti, E.S.G., Duffy, S., Zerbini, F.M., 2017. The diversification of begomovirus populations is predominantly driven by mutational dynamics. Virus Evol 3, vex005.

Moriones, E., Navas-Castillo, J., 2000. *Tomato yellow leaf curl virus*, an emerging virus complex causing epidemics worldwide. Virus Res 71, 123-134.

Navas-Castillo, J., Fiallo-Olivé, E., Sánchez-Campos, S., 2011. Emerging virus diseases transmitted by whiteflies. Annu Rev Phytopathol 49, 219-248.

Nicaise, V., 2014. Crop immunity against viruses: Outcomes and future challenges. Front Plant Sci 5, 660.

Padidam, M., Beachy, R.N., Fauquet, C.M., 1996. The role of AV2 ("precoat") and coat protein in viral replication and movement in tomato leaf curl geminivirus. Virology 224, 390-404.

Padidam, M., Sawyer, S., Fauquet, C.M., 1999. Possible emergence of new geminiviruses by frequent recombination. Virology 265, 218-224.

Rojas, M.R., Hagen, C., Lucas, W.J., Gilbertson, R.L., 2005. Exploiting chinks in the plant's armor: evolution and emergence of geminiviruses. Annu Rev Phytopathol 43, 361-394.

Rojas, M.R., Jiang, H., Salati, R., Xoconostle-Cazares, B., Sudarshana, M.R., Lucas, W.J., Gilbertson, R.L., 2001. Functional analysis of proteins involved in movement of the monopartite begomovirus, tomato yellow leaf curl virus. Virology 291, 110-125.

Rojas, M.R., Macedo, M.A., Maliano, M.R., Soto-Aguilar, M., Souza, J.O., Briddon, R.W., Kenyon, L.A., Rivera-Bustamante, R.F., Zerbini, F.M., Adkins, S., Legg, J.P., Kvarnheden, A., Wintermantel, W.M., Sudarshana, M.R., Peterschmitt, M., Lapidot, M., Martin, D.P., Moriones, E., Inoue-Nagata, A.K., Gilbertson, R.L., 2018. World management of geminiviruses. Annu Rev Phytopathol 56, 637-677.

Rybicki, E.P., 1994. A phylogenetic and evolutionary justification for three genera of *Geminiviridae*. Arch Virol 139, 49-77.

Stenger, D.C., Revington, G.N., Stevenson, M.C., Bisaro, D.M., 1991. Replicational release of geminivirus genomes from tandemly repeated copies: Evidence for rolling-circle replication of a plant viral DNA. Proc Natl Acad Sci USA 88, 8029-8033.

Tavares, E.T., Tatagiba, J.S., Martins, N.F., Silva, F.R., Marinho, V.L.A., Ventura, J.A., Zambolim, E.M., M.T., S.J., 2003. Deciphering the genome of the virus causing "Papaya Sticky Disease". Fitopatol Bras 28, S253-254.

Taylor, L.H., Latham, S.M., Woolhouse, M.E., 2001. Risk factors for human disease emergence. Phil Trans Royal Soc B (Biol Sci) 356, 983-989.

Vyskočilová, S., Tay, W.T., van Brunschot, S., Seal, S., Colvin, J., 2018. An integrative approach to discovering cryptic species within the *Bemisia tabaci* whitefly species complex. Sci Rep 8, 10886.

Ward, B.M., Lazarowitz, S.G., 1999. Nuclear export in plants: Use of geminivirus movement proteins for a cell-based export assay. Plant Cell 11, 1267-1276.

Xavier, C.A.D., 2015. Species diversity and genetic variability of bipartite begomoviruses in the New World, Dep. de Fitopatologia. Universidade Federal de Viçosa, Viçosa, MG, p. 106.

Zhou, X., 2013. Advances in understanding begomovirus satellites. Annu Rev Phytopathol 51, 357-381.

OBJECTIVES

1. Main objectives

Chapter I: To find out whether the divergent begomovirus Sida yellow spot virus (SiYSV) is capable of forming geminate particles.

Chapter II: To perform the biological and molecular characterization of SiYSV.

2. Specific objectives

Chapter I:

i) To infer the three-dimensional structure of the SiYSV CP.

ii) To observe viral particles under a transmission electron microscope.

Chapter II:

i) To determine the host range of SiYSV.

ii) To verify whether *B. tabaci* MEAM1 is able to transmit SiYSV.

iii) To infer another possible insect vector for SiYSV by phylogenetic analysis of the CP.

iv) To verify whether the SiYSV CP interacts with the endosymbiotic bacterial protein GroEL.

i) To identify the subcellular localization of the SiYSV CP.

ii) To verify whether SiYSV has a silencing suppressor protein.

CHAPTER 1

STRUCTURAL ANALYSIS OF SIDA YELLOW SPOT VIRUS (SiYSV) PARTICLES AND ITS CAPSID PROTEIN (CP)

Lima, R.R.P.N., Gomes, R.A.L, Stempkowski, L.A., Zerbini, F.M. Structural analysis of Sida yellow spot virus (SiYSV) particles and its capsid protein (CP).

ABSTRACT

Proteins are fundamental for the structure and function of all organisms. They play important roles in all vital processes in the cell, carrying out chemical reactions, transporting molecules, generating energy, transcribing the genetic information, besides many other roles. All proteins fold in a specific, optimal three-dimensional (3D) structure and their function is intrinsically related to this optimal 3D conformation. Several computational programs have been developed to predict protein 3D structure but none of them achieves the accuracy of AlphaFold, a program released in 2020. AlphaFold is an artificial intelligence program that combines physical and biological knowledge with a deep learning algorithm to predict protein 3D structure. Here, we used AlphaFold to predict the 3D structure of the capsid protein (CP) of a divergent begomovirus, Sida yellow spot virus (SiYSV). The structure was compared with the predicted 3D structures of other typical begomovirus CP's. Ultrathin sections of *Sida acuta* plants infected with SiYSV were examined under a transmission electron microscope to observe the presence, or not, of viral particles. Although the SiYSV CP was structurally very similar to other begomoviruses CPs, viral particles were not observed in infected cells.

INTRODUCTION

Life as we know it is made possible by the existence of four organic macromolecules: nucleic acids, proteins, lipids and carbohydrates. Proteins are the most extensively studied molecules since they are the most abundant in cells and the most structurally and functionally diverse, an important characteristic as it allows them to play a variety of important roles. Their functional diversity enables them to be involved in basically all life processes: metabolism, gene expression, cellular respiration and photosynthesis, cellular communication, assembly of intra- and extracellular structures, protecting cells from pathogens, and many others (Kessel and Ben-Tal, 2018).

Proteins are polymers formed by a set of 20 amino acids which vary in terms of structure and electric charge. The physicochemical properties of amino acids lead to attraction or repulsion between them, which drive the protein chain to fold in a three-dimensional (3D) structure. Thus, the primary sequence of amino acids in the protein chain ultimately determines its 3D folding. The order in which they appear along the chain creates a unique pattern of attractiveness and repulsiveness, which results in a specific (optimal) 3D structure (Kessel and Ben-Tal, 2018; Rasheed et al., 2020).

A protein's 3D structure determines its function (Rasheed et al., 2020). The 3D conformation may expose binding sites, channels and receptors, impacting the way the protein binds to other molecules and its interaction with other proteins to form structural complexes and for carrying out regulatory processes. Therefore, determining the 3D structure of a protein is essential for understanding how it works (Kessel and Ben-Tal, 2018; Rasheed et al., 2020).

Determination of the 3D structure of many proteins has been done through complex methodologies. The most used are X-ray crystallography, nuclear magnetic resonance

spectroscopy, electron microscopy and, recently, cryo-electron microscopy (Abrescia et al., 2012; Richert-Pöggeler et al., 2018).

Viruses are non-cellular life forms which contain a nucleic acid genome and a protein-based capsid, forming the so-called virus "particle". Some viruses also contain a lipid-based envelope encasing the capsid (Koonin et al., 2021). The emergence of viral diseases in the last decades has shed light on the importance of understanding their biology, structure and the interaction between viral and host proteins (McLeish et al., 2019; Zheng et al., 2019). Electron microscopy (EM) provides images of viruses at nanometer scale which are useful for diagnosis and research. For many viruses, the observation of particle structure and size allows preliminary classification at the family level (Richert-Pöggeler et al., 2018).

Advances in cryo-electron microscopy and image processing have improved the determination of the 3D structure of viral capsids. Encapsidation of the viral genome is an essential step of the viral infection cycle, and cryo-EM has been helping to elucidate the mechanisms of capsid assembly and genome encapsidation (Hesketh et al., 2018; Hipp et al., 2017; Richert-Pöggeler et al., 2018).

The family *Geminiviridae* comprises 14 genera of non-enveloped, single-stranded (ss) DNA viruses that infect economically important crops worldwide. Their genome is organized into one or two circular ssDNA components of 2.5-2.8 kb in length, which encode proteins responsible for replication, encapsidation, movement and suppression of host defenses (Fiallo-Olive et al., 2021). A characteristic feature of geminiviruses is the twinned (geminate) morphology of their particles, which consist of two fused T=1 icosahedral capsids forming two hemispheres. At the interface of these hemispheres each capsid loses a pentamer, with the final structure being composed of 110 copies of the capsid protein (CP) (Bennett and Agbandje-McKenna, 2020; Hesketh et al., 2018; Saunders et al., 2020). The CP is the only structural protein encoded by geminiviruses and is also essential for transmission by the insect vector

(Azzam et al., 1994; Briddon et al., 1990; Hohnle et al., 2001). This multifunctional nature of the CP places constraints in its capacity to vary without losing fitness, and in fact the CP is the most conserved protein in the geminivirus genome.

The 3D structure of the capsids of three geminiviruses, African cassava mosaic virus (ACMV), Ageratum yellow mosaic virus (AYVV) and maize streak virus (MSV) have been determined by cryo-EM (Hesketh et al., 2018; Hipp et al., 2017; Zhang et al., 2001). A feature of the geminivirus CP is the signature jelly-roll topology, which is characteristic of the CPs of most viruses with isometric particles (Koonin et al., 2020; Krupovic and Koonin, 2017). A jelly-roll fold consists of eight antiparallel β-sheets organized in two sheets that form the body of the CP (Krupovic and Koonin, 2017). The loops connecting the β-sheets are the most diverse portion of the CP and appear to be important for interaction with the insect vectors that transmit geminiviruses in nature (Cheng and Brooks, 2013; Hesketh et al., 2018).

The core structure of the geminivirus CP (which incluldes the jelly-roll fold) is mostly invariant. However, differences in the N-terminal region allow the CP to adopt the three different conformations needed to build a T=1 particle. These different conformations are especially important at the interface of the two hemispheres, where the interaction between two N-terminal chains (chains H and I) of the CP is critical for particle assembly (Hesketh et al., 2018).

Besides the self-interaction ability of the CP, it also interacts with ssDNA. Capsid assembly is driven by DNA binding, which may be the reason why empty geminiviral particles have never been found in plant infections (Hesketh et al., 2018; Richert-Pöggeler et al., 2018).

All studies on geminivirus particle structure have been done using TEM and cryo-EM. Despite the accuracy of these techniques, they are extremely expensive and time-consuming, such that only around 100,000 protein structures have been determined, which represents a small portion of the billions of already known protein sequences (Jumper et al., 2021; Kuhlman

and Bradley, 2019). Thus, computational approaches have been developed to enable large-scale structural predictions.

There are several computer-based algorithms that can be used to predict a protein 3D structure. In 2021, the AlphaFold program was released (Jumper et al., 2021). AlphaFold combines knowledge of the physical and biological properties of proteins with a deep learning algorithm (Jumper et al., 2021; Senior et al., 2020). It is the first program to use artificial intelligence and deep learning to predict protein 3D structures, and its >90% accuracy has lead scientists to call it "a data-driven revolution" (Thornton et al., 2021).

Whitefly-transmitted geminiviruses (classified in the genus *Begomovirus*) have emerged as important plant pathogens over the last 30 years. Begomoviruses are broadly divided into Old World and New World, with differences in genome organization and protein motifs, including in the CP. Our group has described a large number of begomoviruses, including one infecting the non-cultivated plant *Sida acuta*, named Sida yellow spot virus (SiYSV). SiYSV has a highly divergent CP and several features of OW begomoviruses, such as an AV2-like gene in the virion-sense strand and the presence of conserved motifs that are characteristic of OW begomovirus proteins.

The non-cultivated plant *Sida acuta* is a perennial species which is capable of vegetative propagation. Moreover, despite systematic sampling of SiYSV-infected *S. acuta* plants since 2011, whiteflies have never been observed. Since begomoviruses are capable of establishing a systemic infection without the CP (Sudarshana et al., 1998), we hypothesized that SiYSV may not form geminate particles and is transmitted solely by vegetative propagation of its host. The objectives of this work were to predict the 3D structure of the highly divergent CP encoded by SiYSV using AlphaFold and to determine whether SiYSV can form geminate particles by electron microscopy.

MATERIAL AND METHODS

1. Three-dimensional protein prediction

The three-dimensional (3D) structure of the SiYSV-encoded capsid protein (CP) was predicted using AlphaFold (Jumper et al., 2021; Senior et al., 2020). The 3D structure of the capsid proteins of Ageratum yellow vein virus (AYVV; GenBank accession number X74516), Oxalis yellow vein virus (OxYVV; KM887907), Sida golden yellow spot virus (SGYSV; KX348185), Tomato severe rugose virus (ToSRV; DQ207749) and Apple geminivirus 1 (AGV1; KM386645) were also predicted using AlphaFold to compare and validate the CP structure obtained for SiYSV.

The structure of the SiYSV CP was pairwise aligned to each one of the CPs above using the multiple structure alignment algorithm mTM-align (Dong et al., 2017), and the alignment was observed using the RCSB Protein Data Bank (RCSB PDB) 3D View (Berman et al., 2000).

2. Viral isolates and infectious clones

The viral isolates SiYSV-[BR-Vic26-11] and OxYVV-[BR-1D-11] were obtained from *Sida acuta* plants collected in the municipality of Viçosa, state of Minas Gerais, Brazil, on December 2011 (Godinho, 2014; Xavier, 2015). Infectious clones corresponding to 1.5 and 1.4 copies of the SiYSV-[BR-Vic26-11] DNA-A and DNA-B, respectively (Xavier, 2015), and to 1.4 and 1.6 copies of the OxYVV-[BR-1D-11] DNA-A and DNA-B, respectively (Oliveira, 2021), were used for all experiments.

3. Particle observation by transmission electron microscopy (TEM)

Young *Sida acuta* plants with two to four fully expanded leaves were inoculated by biolistics (Aragão et al., 1996) with the SiYSV and OxYVV infectious clones. Gold particles

coated with 5 µg of DNA-A + DNA-B of each isolate were delivered at 5 psi. As negative controls, four plants were inoculated with gold particles without DNA. Following inoculation, the plants were kept in a greenhouse and symptom development was observed until 28 days post-inoculation (dpi).

At 28 dpi, plants showing characteristic symptoms (Figure 1) were selected. To confirm the infection, total DNA was extracted as described by Doyle and Doyle (1987) and used as a template for PCR using a DNA-A-specific primer pair for SiYSV (SiY-2F, 5'-GAG TTA TCC CTA TGT CCC CC-3'; SiY2-R, 5'-GAG AAT GGC GTC TAT ACC TGG-3') and the begomovirus degenerated primer pair PAL1v1978 and PAR1c496 (Rojas et al., 1993) for OxYVV. The PCR reaction was performed using Taq DNA polymerase (Cellco) and the PCR program consisted of an initial denaturation at 94ºC for 10 min (2 min for OxYVV) followed by 35 cycles at 94ºC for 1 min, 60ºC for 1 min (55ºC for OxYVV) and 72ºC for 2 min with a final extension at 72ºC for 5 min.

Leaf samples of PCR-positive plants were collected and fixated using Karnovsky solution (2% paraformaldehyde and 2.5% glutaraldehyde) followed by 1% osmium tetroxide. Then, samples were dehydrated in a graded ethanol series (30, 50, 70, 80, 95 and 100%) and infiltrated with Spurr resin. Ultrathin sections were mounted on copper grids (200 mesh), stained with 2% uranyl acetate and 1% lead acetate for 20 min each and examined in a FEI Tecnai G2-12 SpiritBiotwin transmission electron microscope at 120 kV, at the Microscopy Center of the Federal University of Minas Gerais (UFMG).

RESULTS

1. Three-dimensional protein prediction

We predicted the 3D structure of the divergent SiYSV CP using AlphaFold and compared it with other typical begomovirus CPs.

Despite the low degree of amino acid sequence similarity, the SiYSV CP is predicted to assume a 3D conformation similar to that of other begomoviruses (Figure 2). In fact, its 3D structure was 66% similar to that of the AYVV CP (Figure 2A), a virus whose 3D structure has been predicted by cryo-electron microscopy. Moreover, it was 75% similar to the SGYMV CP (Figure 2B), the virus that shares the highest amino acid similarity with SiYSV, 67% similar to both ToSRV and OxYVV CP's (Figures 2C and 2D, respectively), viruses used as positive controls throughout this work and 72% similar do AGV1 (Figure 2E), the virus which shares the highest nucleotide similarity with SiYSV.

The 3D structure prediction also indicated that the SiYSV CP assumes a jelly-roll fold with two twisted, 4-stranded β-sheets, typical of most icosahedral viruses (including the other begomoviruses included in the analysis). This core region of the four begomovirus CP's analyzed has a more conserved structure, while their N-terminal regions have a more variable conformation.

These results strongly indicate that the SiYSV CP has the potential to function just as any other begomovirus CP does. Thus, it is not unreasonable to believe that CP subunits can interact with each other and with the viral DNA to assemble into geminate viral particles.

2. Attempts at particle observation by TEM

Based on the results of the 3D structure prediction, we hypothesized that SiYSV may indeed form a geminate viral particle. To confirm this, we observed ultrathin sections of SiYSV-infected leaves under a TEM.

Electron-dense regions that resemble aggregates of viral particles were observed in the nuclei of the OxYVV-infected cells (Figures 3C and 4). The same material was observed outside of the nucleus in a cytoplasmic region closer to the nuclear membrane (Figures 4C and 4D). Images at higher resolution are needed to confirm whether this electron-dense regions are formed by aggregates of viral particles or by condensed nuclear material.

On the other hand, nuclei of SiYSV-infected cells did not show any electron-dense region that could resemble viral particles (Figure 3B). In fact, they are similar to the nuclei of mock-inoculated cells (Figure 3A).

DISCUSSION

The atypical features of the SiYSV-*Sida acuta* pathosystem, which include: (*i*) the highly divergent SiYSV CP, (*ii*) the fact that *Sida acuta* is capable of vegetative propagation, and (*iii*) the lack of whiteflies in the area where the virus has been detected since 2011, lead us to question whether SiYSV actually forms geminate particles. Thus, we performed an *in silico* analysis of the SiYSV CP using Alphafold, and transmission electron microscopy (TEM) analysis of ultrathin sections of SiYSV-infected plants in an attempt to visualize SiYSV particles.

The three-dimensional conformation of a protein is intrinsically related to its function. Knowledge of how proteins fold and the 3D structure they assume is therefore essential to

understand their function and their various biological interactions (Kessel and Ben-Tal, 2018; Lupas et al., 2021; Rasheed et al., 2020).

Our structure predictions and comparisons found TM-scores varying from 66% to 75% between the SiYSV CP and the CP's of AYVV, OxYVV, SGYSV, ToSRV and AGV1. These results indicate a high level of similarity among these proteins, which is surprising considering the very low degree of amino acid sequence similarity among them. Moreover, the core of all predicted CP's was predicted to assume the signature jelly-roll fold and, as expected, most of the differences among the CP's are located in the N-terminal portion of the proteins. Together, these results highlight the importance of considering the 3D structure of viral proteins when trying to infer their function from their amino acid sequence (Krupovic and Koonin, 2017).

The similarity between the SiYSV CP and the typical CP's of the other begomoviruses analyzed indicates that this divergent CP may indeed function like the other begomovirus CPs. Thus, it may have the ability to self-interact and to bind ssDNA for capsid assembly and particle formation.

In cells of OxYVV-infected plants, electron-dense regions were observed inside the nucleus that resemble the aggregates of viral particles encountered in begomovirus infections (Mahmoudieh et al., 2020; Morales et al., 1990). These electron-dense regions appear to have a honeycomb shape as observed in bean golden mosaic virus (BGMV)-infected common bean cells (Kim et al., 1978), although we could not see a clear delimitation between the putative particles or an individual geminate particle. Thus, we cannot claim that the electron-dense regions observed are indeed aggregates of viral particles. However, we observed the same electron-dense regions in the cytoplasm adjacent to the nucleus. Since viral particles are formed in the nucleus and then are shuttled to the cytoplasm so they can be moved to neighboring cells, this observation adds another piece of evidence in favor of the electron-dense regions being comprised of viral particles. It should be noted that OxYVV has a typical begomovirus CP and

is transmitted by whiteflies (Xavier, 2015), which is a clear indication that it forms geminate viral particles.

In contrast to what was observed in the OxYVV-infected cells, no electron-dense regions, or any other type of strucutre that resembled viral particles or aggregates of particles, were observed in SiYSV-infected cells. The failure to visualize SiYSV particles coud be due to a number of reasons. The virus could be present at very low concentrations in the plant, decreasing the chances of an infected cell being present in the ultrathin sections. Previous studies have shown that viral isolates that lack the CP or produce a mutant CP are still infectious but accumulate low levels of ssDNA in cells and display delayed or attenuated symptoms (Fondong, 2013; Harrison et al., 2002; Qin et al., 1998). It is also possible that the virus was present at low concentration at the time of sampling. For example, BGMV and bean dwarf mosaic virus (BDMV) particles were observed by TEM from 8 to 10 days after inoculation of common bean leaves (Kim et al., 1978; Morales et al., 1990), suggesting that 28 dpi may be too late for particle observation, and that tissue samples should be collected at an earlier stage of the infection. However, the timing of viral accumulation could be different in the BGMV/bean pathosystem compared to the SiYSV/*Sida acuta* system. It should be noted that both SiYSV and OxYVV were inoculated in the same host (*Sida acuta*) and the samples were processed at the same point of the infection (28 dpi) for both viruses. In any event, it is important to perform additional studies with SiYSV with samples collected at earlier time points.

Another possibility would be the instability of the SiYSV capsid. It is possible that SiYSV does form particles but that they are unstable and do not tolerate the TEM sample processing. In this regard, significant changes that result in misfolding of capsid proteins can affect its stability and consequently the stability of the viral capsid. Previous studies with ACMV and AYVV revealed the importance of the N-terminal portion of the CP for their self-interaction at the interface of the two hemicapsids and thus for particle assembly. This region

is stabilized by hydrogen bonds, and mutations at two important amino acid residues (R48A and M59D) disrupted the hydrogen bonding between two CP chains, resulting in formation of single icosahedral instead of geminate particles. Also, a mutation at R41 (R41A) disrupted DNA binding and abolished any type of particle formation (Hesketh et al., 2018; Saunders et al., 2020). Since the major differences between the SiYSV CP and the other begomovirus CP's presented here are located in their N-terminal portions, it is possible that a mutation in this region weakens the capsid assembly of SiYSV, leading to capsid instability and disassembly during sample preparation for TEM.

In conclusion, although we were unable to visualize SiYSV particles by TEM, the accumulated evidence suggests that the virus does form geminate particles, although they may be unstable compared to the particles formed by other begomoviruses.

REFERENCES

Abrescia, N.G., Bamford, D.H., Grimes, J.M., Stuart, D.I., 2012. Structure unifies the viral universe. Annu Rev Biochem 81, 795-822.

Aragão, F.J.L., Barros, L.M.G., Brasileiro, A.C.M., Ribeiro, S.G., Smith, F.D., Sanford, J.C., Faria, J.C., Rech, E.L., 1996. Inheritance of foreign genes in transgenic bean (*Phaseolus vulgaris* L.) co-transformed via particle bombardment. Theor Appl Genet 93, 142-150.

Azzam, O., Frazer, J., De La Rosa, D., Beaver, J.S., Ahlquist, P.G., Maxwell, D.P., 1994. Whitefly transmission and efficient ssDNA accumulation of bean golden mosaic geminivirus require functional coat protein. Virology 204, 289-296.

Bennett, A., Agbandje-McKenna, M., 2020. Geminivirus structure and assembly. Adv Virus Res 108, 1-32.

Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., Bourne, P.E., 2000. The Protein Data Bank. Nucleic Acids Res 28, 235-242.

Briddon, R.W., Pinner, M.S., Stanley, J., Markham, P.G., 1990. Geminivirus coat protein gene replacement alters insect specificity. Virology 177, 85-94.

Cheng, S., Brooks, C.L., III, 2013. Viral capsid proteins are segregated in structural fold space. PLoS Comp Biol 9, e1002905.

Dong, R., Peng, Z., Zhang, Y., Yang, J., 2017. mTM-align: an algorithm for fast and accurate multiple protein structure alignment. Bioinformatics 34, 1719-1725.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochem Bull 19, 11-15.

Fiallo-Olive, E., Lett, J.M., Martin, D.P., Roumagnac, P., Varsani, A., Zerbini, F.M., Navas-Castillo, J., 2021. ICTV Virus Taxonomy Profile: *Geminiviridae* 2021. J Gen Virol 102, 001696.

Fondong, V.N., 2013. Geminivirus protein structure and function. Mol Plant Pathol 14, 635-649.

Godinho, M.T., 2014. Coexistência e evolução molecular de populações de begomovírus na planta não-cultivada *Sida acuta*, Dep. de Fitopatologia. Universidade Federal de Viçosa, Viçosa, MG, p. 71.

Harrison, B.D., Swanson, M.M., Fargette, D., 2002. Begomovirus coat protein: serology, variation and functions. Physiol Mol Plant Pathol 60, 257-271.

Hesketh, E.L., Saunders, K., Fisher, C., Potze, J., Stanley, J., Lomonossoff, G.P., Ranson, N.A., 2018. The 3.3 Å structure of a plant geminivirus using cryo-EM. Nature Comm 9, 2369.

Hipp, K., Grimm, C., Jeske, H., Böttcher, B., 2017. Near-atomic resolution structure of a plant geminivirus determined by electron cryomicroscopy. Structure 25, 1303-1309.e1303.

Hohnle, M., Hofer, P., Bedford, I.D., Briddon, R.W., Markham, P.G., Frischmuth, T., 2001. Exchange of three amino acids in the coat protein results in efficient whitefly transmission of a nontransmissible *Abutilon mosaic virus* isolate. Virology 290, 164-171.

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S.A.A., Ballard, A.J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., Back, T., Petersen, S., Reiman, D., Clancy, E., Zielinski, M., Steinegger, M., Pacholska, M., Berghammer, T., Bodenstein, S., Silver, D., Vinyals, O., Senior, A.W., Kavukcuoglu, K., Kohli, P., Hassabis, D., 2021. Highly accurate protein structure prediction with AlphaFold. Nature 596, 583-589.

Kessel, A., Ben-Tal, N., 2018. Introduction to Proteins: Structure, Function, and Motion, 2nd ed. Chapman and Hall/CRC, New York.

Kim, K.S., Shock, T.L., Goodman, R.M., 1978. Infection of *Phaseolus vulgaris* by bean golden mosaic virus: ultrastructural aspects. Virology 89, 22-33.

Koonin, E.V., Dolja, V.V., Krupovic, M., Kuhn, J.H., 2021. Viruses defined by the position of the virosphere within the replicator space. Microbiol Mol Bio Rev 85, e00193-00120.

Koonin, E.V., Dolja, V.V., Krupovic, M., Varsani, A., Wolf, Y.I., Yutin, N., Zerbini, F.M., Kuhn, J.H., 2020. Global organization and proposed megataxonomy of the virus world. Microbiol Mol Bio Rev 84, e00061-00019.

Krupovic, M., Koonin, E.V., 2017. Multiple origins of viral capsid proteins from cellular ancestors. Proc Natl Acad Sci U S A 114, E2401-E2410.

Kuhlman, B., Bradley, P., 2019. Advances in protein structure prediction and design. Nat Rev Mol Cell Bio 20, 681-697.

Lupas, A.N., Pereira, J., Alva, V., Merino, F., Coles, M., Hartmann, M.D., 2021. The breakthrough in protein structure prediction. Biochem J 478, 1885-1890.

Mahmoudieh, M., Noor, M.R.M., Harikrishna, J.A., Othman, R.Y., 2020. Identification and characterization of Ageratum yellow vein Malaysia virus (AYVMV) and an associated

betasatellite among begomoviruses infecting Solanum lycopersicum in Malaysia. J Appl Genet 61, 619-628.

McLeish, M.J., Fraile, A., García-Arenal, F., 2019. Evolution of plant-virus interactions: Host range and virus emergence. Curr Opin Virol, 50-55.

Morales, F., Niessen, A., Ramirez, B., Castaño, M., 1990. Isolation and partial characterization of a geminivirus causing bean dwarf mosaic. Phytopathology 80, 96-101.

Oliveira, P.G.S., 2021. Obtenção e caracterização de clones infecciosos de um begomovírus isolado de *Sida acuta*, Dep. de Bioquímica e Biologia Molecular. Universidade Federal de Viçosa, Viçosa, MG.

Qin, S., Ward, B.M., Lazarowitz, S.G., 1998. The bipartite geminivirus coat protein aids BR1 function in viral movement by affecting the accumulation of viral single-stranded DNA. J Virol 72, 9247-9256.

Rasheed, F., Markgren, J., Hedenqvist, M., Johansson, E., 2020. Modeling to understand plant protein structure-function relationships-implications for seed storage proteins. Molecules 25, 873.

Richert-Pöggeler, K.R., Franzke, K., Hipp, K., Kleespies, R.G., 2018. Electron microscopy methods for virus diagnosis and high resolution analysis of viruses. Front Microbiol 9, 3255.

Rojas, M.R., Gilbertson, R.L., Russell, D.R., Maxwell, D.P., 1993. Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. Plant Dis 77, 340-347.

Saunders, K., Richardson, J., Lawson, D.M., Lomonossoff, G.P., 2020. Requirements for the packaging of geminivirus circular single-stranded DNA: Effect of DNA length and coat protein sequence. Viruses 12, 1235.

Senior, A.W., Evans, R., Jumper, J., Kirkpatrick, J., Sifre, L., Green, T., Qin, C., Žídek, A., Nelson, A.W.R., Bridgland, A., Penedones, H., Petersen, S., Simonyan, K., Crossan, S., Kohli, P., Jones, D.T., Silver, D., Kavukcuoglu, K., Hassabis, D., 2020. Improved protein structure prediction using potentials from deep learning. Nature 577, 706-710.

Sudarshana, M.R., Wang, H.L., Lucas, W.J., Gilbertson, R.L., 1998. Dynamics of bean dwarf mosaic geminivirus cell-to-cell and long-distance movement in *Phaseolus vulgaris* revealed, using the green fluorescent protein. Mol Plant-Microbe Int 11, 277-291.

Thornton, J.M., Laskowski, R.A., Borkakoti, N., 2021. AlphaFold heralds a data-driven revolution in biology and medicine. Nature Med 27, 1666-1669.

Xavier, C.A.D., 2015. Species diversity and genetic variability of bipartite begomoviruses in the New World, Dep. de Fitopatologia. Universidade Federal de Viçosa, Viçosa, MG, p. 106.

Zhang, W., Olson, N.H., Baker, T.S., Faulkner, L., Agbandje-McKenna, M., Boulton, M.I., Davies, J.W., McKenna, R., 2001. Structure of the *Maize streak virus* geminate particle. Virology 279, 471-477.

Zheng, N., Wang, K., Zhan, W., Deng, L., 2019. Targeting virus-host protein interactions: Feature extraction and machine learning approaches. Curr Drug Metab 20, 177-184.

Figure legends

Figure 1. Symptomatic *Sida acuta* plants following biolistic inoculation with infectious clones of Sida yellow spot virus (SiYSV) and Oxalis yellow vein virus (OxYVV). Images were obtained at 28 days post-inoculation.

Figure 2. Alphafold-predicted 3D structures of the capsid proteins (CP) of Ageratum yellow vein virus (AYVV), Sida golden yellow spot virus (SGYSV), tomato severe rugose virus (ToSRV), Oxalis yellow vein virus (OxYVV) and Sida yellow spot virus (SiYSV). Comparisons between the SiYSV CP and the other predicted CP's were made with the mTM-align algorithm. (A) AYVV and SiYSV; (B) SGYSV and SiYSV; (C) ToSRV and SiYSV; (D) OxYVV and SiYSV; (E) AGV1 and SiYSV.

Figure 3. Transmission electron microscopy images of ultrathin section from *Sida acuta* cells. (A) Mock-inoculated plants; (B) Plants inoculated with Sida yellow spot virus (SiYSV); (C) Plants inoculated with Oxalis yellow vein virus (OxYVV). Scale bars are indicated in each image. CW: cell wall; C: cytoplasm; NM: nuclear membrane; N: nucleus; Nu: nucleolus; EDA: electron-dense aggregates.

Figure 4. Transmission electron microscopy images of ultrathin section from *Sida acuta* cells infected with Oxalis yellow vein virus (OxYVV). Red arrows indicate electron-dense aggregates (EDA) resembling geminiviruses particles occurring outside of the nucleus, and white arrows indicate the same structures inside the nucleus. Scale bars are indicated in each image. CW: cell wall; C: cytoplasm; NM: nuclear membrane; N: nucleus; Nu: nucleolus.

Figure 1

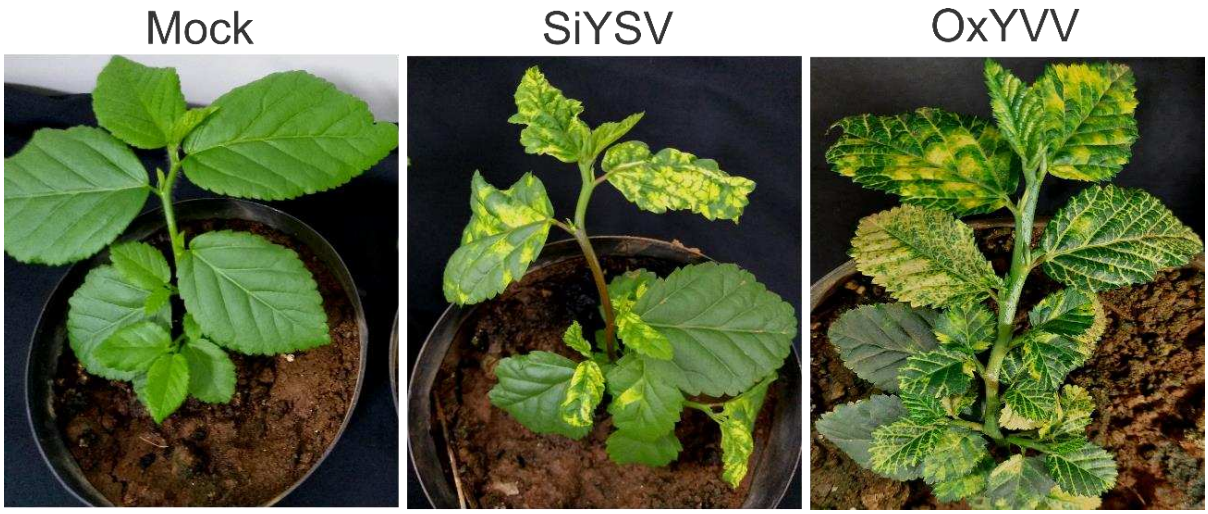


Figure 2

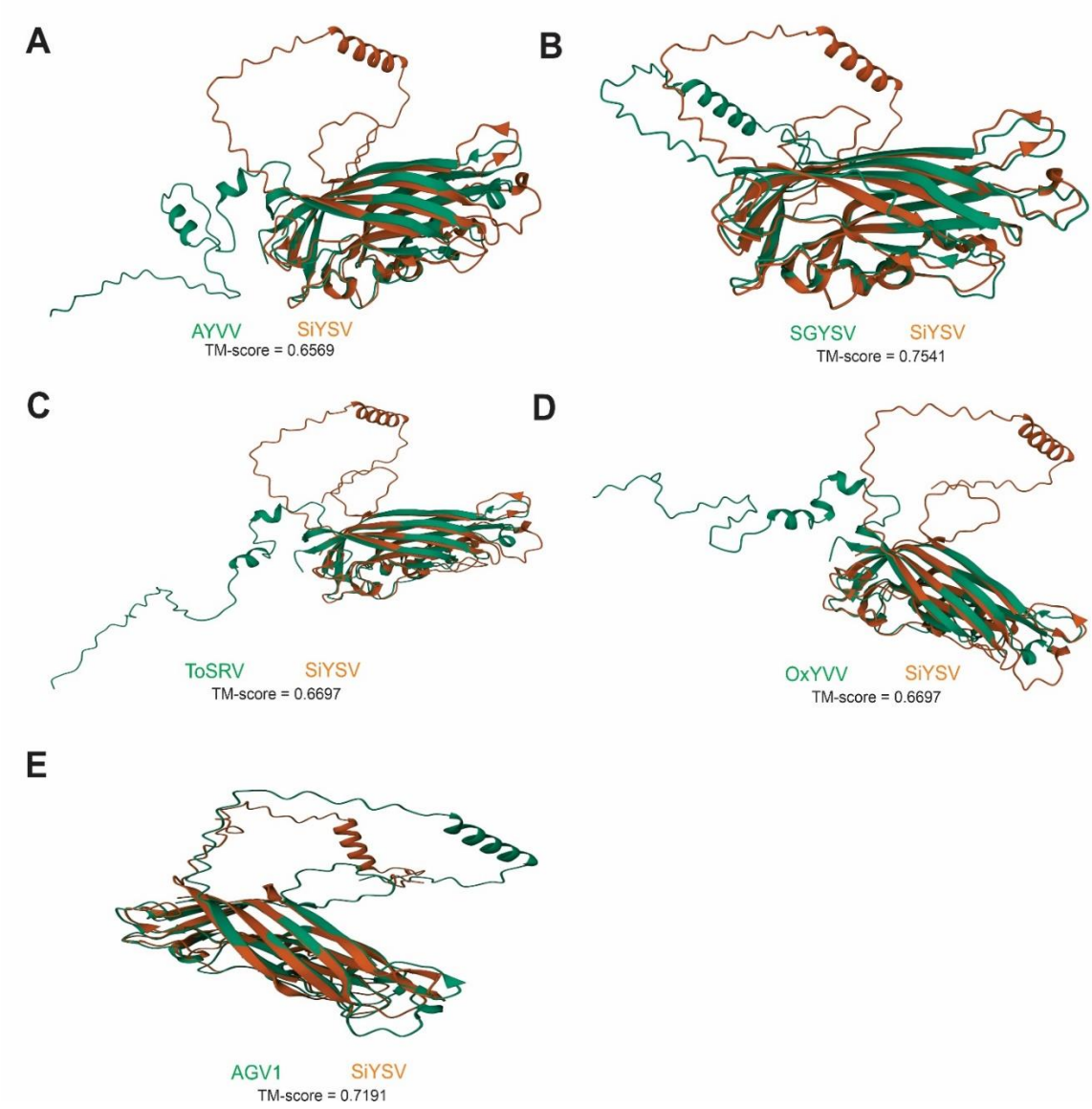

A
AYVV SiYSV
TM-score = 0.6569
B
SGYSV SiYSV
TM-score = 0.7541
C
ToSRV SiYSV
TM-score = 0.6697
D
OxYVV SiYSV
TM-score = 0.6697
E
AGV1 SiYSV
TM-score = 0.7191

Figure 3

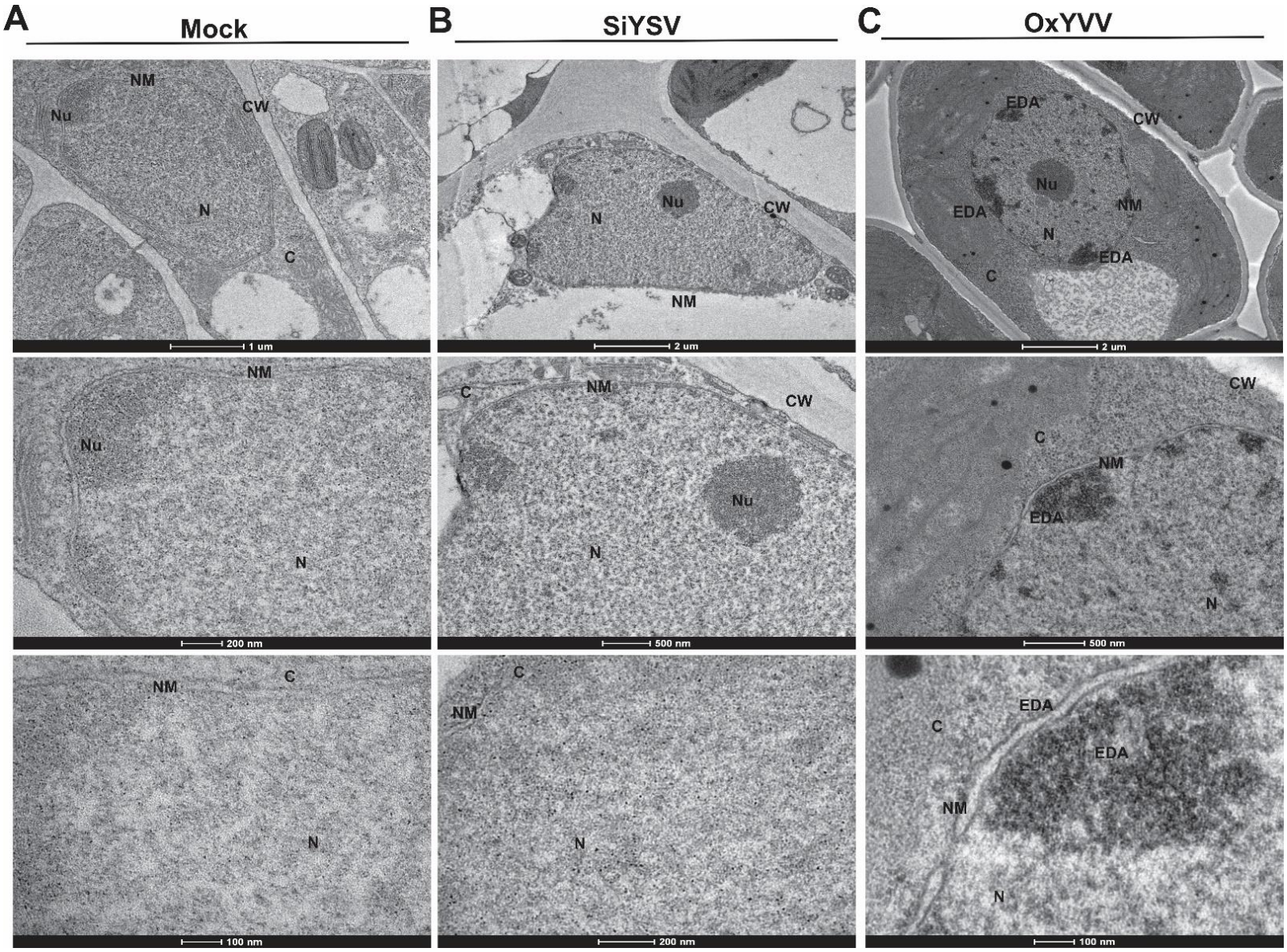
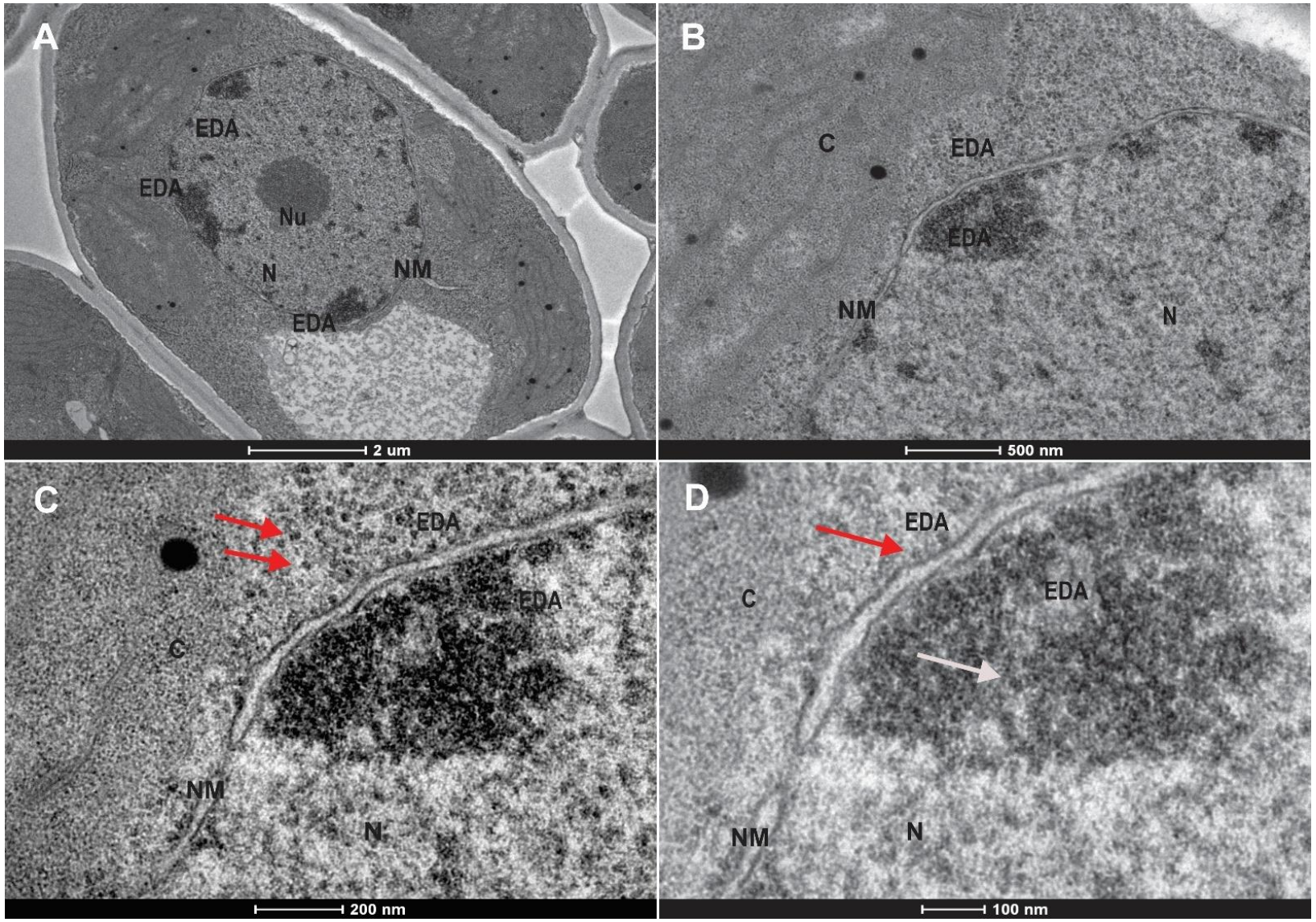


Figure 4


A
EDA
EDA
Nu
N
NM
EDA
2 um
B
C
EDA
EDA
NM
N
500 nm
C
EDA
EDA
C
NM
N
200 nm
D
EDA
C
EDA
NM
N
100 nm

CHAPTER 2

BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF SIDA YELLOW SPOT VIRUS (SiYSV)

Lima, R.R.P.N., Stempkowski, L.A., Reis, A.C.S., Silva, J.P., Orílio, A.F., Zerbini, F.M. Biological and molecular characterization of Sida yellow spot virus (SiYSV).

ABSTRACT

Begomoviruses are among the most important group of plant pathogens. They are circular, single-stranded DNA viruses that encapsidate their genomes in geminate, quasi-icosahedral particles. These viruses encode multifunctional proteins involved in replication, gene expression, suppression of host defenses, movement and encapsidation, The begomovirus capsid protein (CP), besides its major role as the virus single structural protein, is also responsible for viral acquisition and transmission by the insect vector. It protects the viral particle of degradation inside the insect vector by physically interacting with a protein present in the insect hemolymph named GroEL. Usually, the CP is the most conserved begomoviral protein, however a new begomovirus, named Sida yellow spot virus (SiYSV) has been described with a highly divergent CP. Here, we carried out the biological and molecular characterization of SiYSV. First, we performed a host range assay and a whitefly transmission assay. SiYSV infected the cultivated host *Abelmoschus esculentus* (okra), but *B. tabaci* MEAM1 was not able to transmit the virus. We then investigated by bimolecular fluorescence complementation (BiFC) whether the SiYSV CP would interact with GroEL. The analysis indicated a lack of interaction between these two proteins. Finally, the subcellular localization of SiYSV-encoded proteins was assessed on a laser scanning confocal microscope and their ability to suppress RNA silencing was analyzed. We showed that these proteins had the same pattern of localization expected for proteins from typical begomoviruses, but they do not show any silencing suppression activity. These results indicate that the SiYSV-encoded proteins can function just as those from other begomoviruses, and that the inability of *B. tabaci* to transmit SiYSV may be due to the lack of interaction between its CP and GroEL.

INTRODUCTION

Geminiviruses are considered one of the most devastating groups of plant pathogens, causing great losses in many economically important crops such as beans, cassava, cotton, cucurbits, maize, peppers and tomato (Legg and Fauquet, 2004; Moriones and Navas-Castillo, 2000; Navas-Castillo et al., 2011; Rojas et al., 2018). The family is comprised of 14 genera (Fiallo-Olive et al., 2021) and among them the genus *Begomovirus* has the highest number of species and is the most economically important.

Begomoviruses have one or two genomic components of circular, single-stranded DNA (ssDNA) of 2,600-2,800 nucleotides (nt) in length, encapsidated in geminate, quasi-icosahedral particles. They are transmitted by whiteflies of the *Bemisia tabaci* species complex (Hemiptera: Aleyrodidae) in a persistent-circulative manner (Czosnek et al., 2017; Fiallo-Olive et al., 2021; Hogenhout et al., 2008). According to their genome architecture and phylogenetic relationships, begomoviruses are divided into two major groups: New World (NW - the Americas) and Old World (OW - Africa, Asia and Europe) (Briddon et al., 2010; Rybicki, 1994). Begomoviruses from the NW are predominantly bipartite, and their genome components are named DNA-A and DNA-B, while the OW begomoviruses can be monopartite or bipartite and are frequently associated with satellite DNA molecules (Zhou, 2013).

In general, plant viruses have small genomes and thus codify a limited number of proteins. Consequently, these proteins are usually multifunctional and often interact with each other (Wang et al., 2022). Some geminiviruses (eg, mastreviruses) encode only four proteins, and bipartite begomoviruses encode from seven to nine (five to seven in the DNA-A and two in the DNA-B). In the DNA-A, the capsid protein (CP) and the AV2 protein are encoded in the virion-sense strand (AV2 is only encoded by OW begomoviruses), while a replication-associated protein (Rep), a transcriptional activator protein (TrAP), a replication enhancer

protein (REn), a pathogenicity factor (AC4) and, in some viruses, an AC5 protein, are encoded in the complementary-sense strand (Fondong, 2013; Hanley-Bowdoin et al., 1999; Li et al., 2015). The DNA-B encodes a nuclear shuttle protein (NSP) and a movement protein (MP) (Fondong, 2013). Rep is responsible for the initiating step in rolling-circle replication of the viral genome and modulation of the cell cycle, activating expression of hosts factors involved in DNA replication (Hanley-Bowdoin et al., 1999). Trap activates the transcription of CP and NSP, and it is also involved in suppression of RNA silencing. Ren is not essential for replication, but its interaction with Rep makes viral replication more efficient and enhances DNA accumulation several fold. It is also related to symptom development. AC4 is a symptom determinant and an RNA silencing suppressor, and is also involved in viral movement. Likewise, AV2 is also involved in movement and is a suppressor of host defenses.

The CP is also a multifunctional protein and besides encapsidation it is involved in ssDNA binding, shuttling of viral DNA in and out of the nucleus and viral transmission by the insect vector (Czosnek et al., 2017; Fondong, 2013; Harrison et al., 2002; Kunik et al., 1998; Rojas et al., 2001). It mediates vector specificity and controls virus transport through the insect body. When virus particles reach the hemolymph, the CP interacts with a GroEL chaperone homologue produced by endosymbiotic bacteria that live inside the insect body in specific cells called bacteriosomes (Czosnek et al., 2017).

The different *B. tabaci* species host the obligatory primary endosymbiont *Portiera aleyrodidarum* and different sets of facultative secondary endosymbionts, among them *Arsenophonus, Cardinium, Fritchea, Hamiltonella, Ricketsia* and *Wolbachia* spp. These bacteria secret GroEL homologs in the insect's gut where they physically interact with the viral CP, protecting the virus from degradation by proteases and nucleases present in the hemolymph (Czosnek et al., 2017; Harrison et al., 2002; Hogenhout et al., 2008).

Important sequences for vector transmission have been mapped to the central and the N-terminal regions of the CP, where a nuclear localization signal (NLS) is located. This region seems to be required for interaction between the CP and GroEL and thus for whitefly transmission efficiency (Fondong, 2013; Hogenhout et al., 2008; Morin et al., 1999). Anything that disrupts this interaction results in degradation of the viral particles (Hogenhout et al., 2008).

The CP is the most conserved begomovirus protein (Harrison et al., 2002). However, a bipartite begomovirus with a highly divergent CP, Sida yellow spot virus (SiYSV), was described infecting the non-cultivated plant *Sida acuta* (Xavier, 2015). Although phylogenetic analyses based on the DNA-A, DNA-B and the Rep gene clustered SiYSV with NW begomoviruses, it has several features of OW begomoviruses, such as longer components of approximately 2800 nt, an AV2 gene and some conserved protein motifs characteristic of OW begomoviruses, including in the CP (Xavier, 2015).

Knowledge of a virus biology is important to understand its life cycle, the dynamics of the infectious process and its epidemiology, with scientific and economic implications. Thus, the objective of this work was to conduct the biological and molecular characterization of the divergent begomovirus Sida yellow spot virus.

MATERIAL AND METHODS

1. Viral isolates, plant material and growth conditions

The viral isolates SiYSV-[BR-Vic26-11] and Oxalis yellow vein virus (OxYVV)-[BR-1D-11] were obtained from *Sida acuta* plants in the municipality of Viçosa, state of Minas Gerais (MG), Brazil, collected on December 2011 (Godinho, 2014; Xavier, 2015). The isolate BR-Bi2-99 of tomato yellow spot virus (ToYSV) was obtained from a tomato plant collected in the municipality of Bicas, MG, in 1999 (Ambrozevicius et al., 2002). Infectious clones

corresponding to 1.5 and 1.4 copies of the SiYSV-[BR-Vic26-11] DNA-A and DNA-B, respectively (Xavier, 2015), to 1.4 and 1.6 copies of the OxYVV-[BR-1D-11] DNA-A and DNA-B, respectively (Oliveira, 2021), and to 1.2 copies of the DNA-A and DNA-B of ToYSV-[BR-Bi2-99] (Andrade et al., 2006) were used in the experiments.

Nicotiana benthamiana plants were maintained in a growth chamber at 22ºC under long-day conditions (16 h light/8 h dark) and *Sida acuta* plants were kept in a greenhouse.

2. Host range assay

A host range assay was conducted using plants of the families Fabaceae, Malvaceae and Solanaceae. Young plants with two to four fully expanded leaves were inoculated by biolistics (Aragão et al., 1996) using the SiYSV infectious clones. As a positive control of the biolistic procedure, two to four plants of each species were inoculated with ToYSV. Gold particles coated with 5 μg of each SiYSV or ToYSV DNA components were delivered at 5 psi. As negative controls, two to four plants of each species were inoculated with gold particles without DNA. After inoculation, the plants were kept in a greenhouse and symptom development was observed until 28 days post-inoculation (dpi). To confirm the infection, total DNA was extracted as described by Doyle and Doyle (1987) and used as a template for PCR using a DNA-A-specific primer pair (SiY-2F and SiY2-R; Table 1) for SiYSV and a degenerate primer pair (PAL1v1978 and PAR1c496; Rojas et al., 1993) for ToYSV. The PCR reaction was performed using Taq DNA polymerase (Cellco) and the PCR program consisted of an initial denaturation step at 94ºC for 10 min, followed by 35 cycles at 94ºC for 1 min, 60ºC for 1 min (55ºC for ToYSV) and 72ºC for 2 min with a final extension at 72ºC for 5 min.

3. Whitefly transmission assay

Colonies of aviruliferous *B. tabaci* Middle East-Asia Minor 1 (MEAM1) whiteflies were maintained in cabbage plants (*Brassica oleraceae*) inside insect-proof cages kept in a growth chamber. Adult whiteflies were collected and transferred to individual *Sida acuta* plants infected with OxYVV (used as positive control) or SiYSV, both inoculated by biolistics. After a 72 h acquisition access period (AAP), 30 whiteflies were collected and transferred to young, healthy *S. acuta* plants, where they were maintained for another 72 h, corresponding to the inoculation access period (IAP). Eleven plants were inoculated with OxYVV and 65 plants with SiYSV. As a negative control, six *S. acuta* plants were exposed to a 72 h IAP with 30 aviruliferous whiteflies. After whitefly elimination with insecticide, the plants were transferred to a greenhouse and kept for symptom observation until 48 dpi. After this period, leaf samples were collected and used for total DNA extraction (Doyle and Doyle, 1987) and the DNA was used as a template for PCR amplification, as mentioned in item 2, using specific primers for SiYSV and the degenerate primer pair PAL1v1978 and PAR1c496 for OxYVV. The procedure is outlined in Figure 1.

4. DNA extraction of *B. tabaci* MEAM1 and identification of bacterial endosymbionts

DNA was extracted from 10 individual whiteflies following a modified Chelex protocol (Walsh et al., 1991). Each whitefly was ground and homogenized in 30 µl of 5% Chelex solution in a 0.6 ml tube. The tube was vortexed for 30 seconds and then incubated at 56ºC for 15 min and at 99ºC for 8 min. After a 5 min centrifugation at 14,000 rpm, the supernatant was collected and kept at -20ºC for a maximum period of 7 days.

These extracted DNAs were used for the screening of the whitefly primary bacterial endosymbiont, *Portiera*, and all six secondary endosymbionts (*Arsenophonus*, *Cardinium*, *Fritchea*, *Hamiltonella*, *Rickettsia* and *Wolbachia*,) using genus-specific primers targeting the

16S and 23S rDNA genes (Table 2). PCR cycling was performed as described above for the host range assay.

Portiera and *Hamiltonella* were selected for the investigation of a possible interaction of their GroEL homologs with the SiYSV CP. The GroEL coding sequences were obtained from GenBank (access numbers EU435142 and AF130421, respectively). One primer pair was constructed for each gene including cloning sites for the pENTR11 vector (Table 1). The coding sequences were amplified by PCR, purified with the Illustra GFX PCR DNA and Gel Band Purification kit (Millipore Sigma) and digested with BamHI and XhoI (Promega). The digested PCR products were inserted into pENTR11, previously digested with BamHI/XhoI, using T4 DNA ligase (New England BioLabs) and the resulting plasmids were transformed into eletrocompetent *E. coli* DH5α. Plasmid DNA was purified using the Wizard Plus Minipreps DNA Purification System (Promega) and the obtained constructs were confirmed by restriction enzyme digestion, PCR amplification and sequencing. The recombinant entry plasmids were transferred to the destination vectors pSITE-nEYFP-N1 and pSITE-cEYFP-N1 by recombination using Gateway LR Clonase II Enzime mix (Invitrogen) and cloned into *E. coli* DH5α. Plasmid DNA was purified, and recombinant plasmids were confirmed by restriction endonuclease digestion and PCR amplification.

5. Comparison of capsid protein sequences

A total of 17 geminivirus capsid protein sequences were aligned with the CP sequence of SiYSV. These sequences belong to the three existing maldoviruses and 14 (four OW and 10 NW) begomoviruses (Table 3). The sequences were aligned using the MUSCLE algorithm implemented in MEGA X (Kumar et al., 2018).

6. Phylogenetic analysis

To infer potential vectors of SiYSV, a phylogenetic analysis was conducted based on the amino acid sequences of the CP. A data set containing 62 viruses from all 14 genera of the family *Geminiviridae* was used for the analysis (Table 3). The sequences were aligned using the MAFFT algorithm (Katoh et al., 2012) implemented on the program RaxML (Stamatakis, 2006) and the phylogenetic tree was constructed using the maximum likelihood method.

7. Plasmid constructs for subcellular localization, interaction and silencing suppression assays

The coding sequences of each SiYSV-encoded protein were amplified by PCR using the primers listed on Table 1. The primers included cloning sites for the pENTR11 vector (Invitrogen). The PCR products were purified using the Illustra GFX PCR DNA and Gel Band Purification kit (Millipore Sigma). Then, the amplified fragments of the Rep, REn, AC4, CP and AV2 genes were digested with BamHI and XhoI (Promega), while BamHI and EcoRV (Promega) were used to digest the amplified TrAP fragment. The digested PCR products were inserted into the entry vector pENTR11 (previously digested by a combination of BamHI/XhoI or BamHI/EcoRV enzymes) using T4 DNA ligase (New England BioLabs) and the resulting plasmids were transformed into eletrocompetent *Escherichia coli* DH5α. Plasmid DNA was purified using the Wizard Plus Miniprep DNA Purification System (Promega). All obtained constructs were confirmed by restriction enzyme digestion, PCR amplification and sequencing. The resulting entry plasmids were then transferred to different destination vectors by recombination using Gateway LR Clonase II Enzime mix (Invitrogen), and the recombinant destination vectors were transformed into *E. coli* DH5α. Plasmid DNA was purified, and the recombinant plasmids were again confirmed by restriction endonuclease digestion and PCR amplification. All reactions were performed following the manufacture's recommendations.

For the subcellular localization assay, each coding region was transferred from the entry vector pENTR11 to the pk7FWG2 vector, generating the following recombinant constructs: pk7FWG2:Rep (Rep-GFP), pk7FWG2:REn (REn-GFP), pk7FWG2:TrAP (TrAP-GFP), pk7FWG2:AC4 (AC4-GFP), pk7FWG2:CP (CP-GFP) and pk7FWG2:AV2 (AV2-GFP).

The pSITE-nEYFP-N1 and pSITE-cEYFP-N1 vectors, which contain the N-terminal (nYFP) and C-terminal (cYFP) region of YFP, respectively, were used for bimolecular fluorescence complementation (BiFC) experiments. The SiYSV CP cloned into pENTR11 was transferred to pSITE-nEYFP-N1 and pSITE-cEYFP-N1, resulting in CP-nYFP and CP-cYFP.

Finally, for the silencing suppression assay, the coding regions of TrAP, AC4 and CP cloned into pENTR11 were recombined into the pART27-HA vector.

8. Agroinfiltration

All subcellular localization, silencing suppression and interaction assays were performed by transient protein expression in *N. benthamiana*. *Agrobacterium tumefaciens* strain GV3101 cells were transformed with the constructs of interest using standard methods (Martin et al., 2009). Transformed cells were grown in LB medium supplemented with specifics antibodies for 48 h at 28ºC. Then, cells were resuspended in infiltration buffer (10 mM $MgCl_2$, 10 mM MES, 150 µM acetosyringone) for a final OD_{600} of 1.0 for both individual and co-infiltrated constructs. Cells were kept at room temperature for 1 to 2 h and then agroinfiltration was performed at the abaxial surface of leaves using a 1 ml syringe without the needle. The plants were kept in a growth chamber at 22ºC under long-day conditions (16 h light/8 h dark) throughout the experiment.

9. Bimolecular fluorescence complementation (BiFC) assay

Interaction between the SiYSV CP and the endosymbiont chaperonin GroEL was investigated in *N. benthamiana* using a BiFC assay.

The pSITE-nEYFP-N1 and pSITE-cEYFP-N1 vectors containing the coding sequence of SiYSV CP and the GroEL gene of the endosymbionts *Portiera* and *Hamiltonella* were used to transform *A. tumefaciens* GV3101 (Wise et al., 2006).

N. benthamiana plants with two to four expanded leaves were agroinfiltrated with the following combinations: pSITE-nEYFP-N1-PGroEL + pSITE-cEYFP-CP, pSITE-nEYFP-N1-HGroEL + pSITE-cEYFP-CP, pSITE-nEYFP-N1-CP + pSITE-cEYFP-PGroEL, and pSITE-nEYFP-N1-CP + pSITE-cEYFP-HGroEL. Agroinfiltration of the empty pSITE-nEYFP-N1 + pSITE-cEYFP-CP was used as a negative control, and the nuclear-localized protein AtWWP1 (Calil et al., 2018) was used as a control of the inoculation. All combinations were co-inoculated with the potyvirus silencing suppressor protein HC-Pro (Anandalakshmi et al., 1998). Fluorescence was observed at 48 and 72 h in a LSM 510 META laser scanning confocal microscope (Carl Zeiss). A wavelength of 488 nm was used for YFP excitation, and emission was detected using a 500-530 nm filter. Excitation of mCherry was at 540 nm and emission was detected at 608-680 nm. All collected images were analyzed with the Zeiss LSM Image Browser 4.2 software.

10. Subcellular localization assay

Four-week-old *N. benthamiana* leaves were co-infiltrated with *A. tumefaciens* GV3101 cells expressing each construct of interest, the nuclear-localized protein AtWWP1 (Calil et al., 2018), the membrane-localized protein AtPIP2A (Cutler et al., 2000), both fused to mCherry, and the potyvirus silencing suppressor HC-Pro (Anandalakshmi et al., 1998). Fluorescence was observed at 2 and 3 days post-infiltration (dpi) under a LSM 510 META laser scanning confocal

microscope (Carl Zeiss). A wavelength of 488 nm was used for YFP excitation, and emission was detected using a 500-530 nm filter. Excitation of mCherry was at 540 nm and emission was detected at 608-680 nm. All collected images were analyzed with the Zeiss LSM Image Browser 4.2 software.

11. Silencing suppression assay

Colonies of *A. tumefaciens* GV3101 were transformed with constructs expressing AC4, TrAP and CP. In this experiment, the empty vector pART27-HA:Ø and a vector expressing HC-Pro (pK7WG2:HC-Pro) were used as negative and positive controls, respectively. Each culture was co-infiltrated in *N. benthamiana* plants together with the construct pK7WG2:GFP, which directs the expression of GFP in the absence of RNA silencing (Johansen and Carrington, 2001; Llave et al., 2000). Leaves were observed under UV light at 3 and 5 dpi.

RESULTS

1. Host range of SiYSV

To determine whether SiYSV is capable of infecting hosts other than *S. acuta*, several plant species belonging to the families Solanaceae and Malvaceae were inoculated by biolistics (Table 4).

SiYSV could not infect the model plant *N. benthamiana* nor *N. tabacum* TNN, and neither tomato (*Solanum lycopersicum*) or cotton (*Gossypium hirsutum*) plants. However, SiYSV successfully infected okra plants (*Abelmoschus esculentum*), causing yellow mosaic and leaf deformation (Figure 2).

2. Whitefly transmission

As the CP of SiYSV is highly divergent, we hypothesized that this virus may not be transmitted, or transmitted with low efficiency, by the whitefly vector. To test this hypothesis, a transmission experiment was conducted using viruliferous *B. tabaci* MEAM1 and long (72 h) AAP/IAP.

Oxalis yellow vein virus (OxYVV), which has a typical begomovirus CP, was efficiently transmitted to young *S. acuta* plants, causing symptoms of yellow mosaic and vein yellowing (Figure 3). On the other hand, SiYSV was not detected, either by symptom observation or PCR, in any of the 65 plants in which the whiteflies that fed on SiYSV-infected plants were maintained (Table 5). Therefore, we concluded that SiYSV is not transmitted by *B. tabaci* MEAM1.

3. Interaction between SiYSV-CP and GroEL

First, the endosymbionts occurring in individuals of *B. tabaci* MEAM1 colonies used throughout this work were identified by PCR as *Portiera, Hamiltonella*, *Rickettsia*, *Wolbachia* and *Fritchea* (data not shown). The primary endosymbiont *Portiera* and the secondary endosymbiont *Hamiltonella* were chosen for the interaction assay. The complete coding sequence of *Portiera*'s and *Hamiltonella*'s GroEL gene was cloned into pENTR11 followed by recombination into pSITE-nEYFP-N1 and pSITE-cEYFP-N1. The same procedures were performed for the SiYSV CP. The recombinant constructs were transiently expressed in *N. benthamiana* leaves.

The yellow fluorescence resulting of the complementation of nYFP and cYFP was not observed in any treatment (Figure 4), indicating that the SiYSV CP did not interact either with *Portiera*'s or with *Hamiltonella*'s GroEL.

4. Capsid protein (CP) sequence comparisons

Even though *B. tabaci* MEAM1 is the predominant vector of begomoviruses in Brazil, *B. tabaci* Mediterranean (MED) is being increasingly detected in open fields (Moraes et al., 2018; Xavier et al., 2021). Previous studies have shown that at amino acid residue 93 located between two conserved regions in the CP, GCEGPCKVQS and LYMACTHASN, the *B. tabaci* MED-non transmissible viruses have a valine, while *B. tabaci* MED-transmissible viruses have an isoleucine (Gautam et al., 2022). An alignment of the CP sequence of SiYSV with 17 other geminiviruses, including maldoviruses and begomoviruses, showed that SiYSV has a valine at residue 93 (Figure 5), which is an indicative that this virus may not be transmitted by *B. tabaci* MED as well.

The region responsible for the interaction between CP and GroEL has been mapped to the N-terminal portion of the protein (Morin et al., 1999). It was reported that the substitution R19L disrupts the interaction between these two proteins and prevents TYLCV transmission (Hogenhout et al., 2008). The CP sequence alignment showed that SiYSV has an isoleucine instead of an arginine at residue 19 (Figure 5), which is consistent with the lack of interaction between the SiYSV CP and GroEL (Figure 4) and would explain why this virus is not transmitted by *B. tabaci* MEAM1 (Table 5).

5. Phylogenetic inference of an insect vector

Geminiviruses transmitted by the same vector have related CP's (Bahder et al., 2016; Briddon et al., 1990). Since SiYSV was not transmitted by *B. tabaci* MEAM1, a phylogenetic tree based on the deduced amino acid sequence of the CP of 62 geminiviruses, representing all 14 genera in the family, was constructed to infer a possible insect vector for SiYSV.

The phylogenetic tree (Figure 6) shows that geminiviruses form clusters corresponding to each genus of the family *Geminiviridae*. These well supported clusters have a clear

association with the respective insect vector. However, an exception occurs with SiYSV, which belongs to the genus *Begomovirus* but whose CP is more closely related to those of members of the genus *Maldovirus*. Unfortunately, the natural vector of these viruses has not yet been identified (Roumagnac et al., 2021).

6. Subcellular localization of SiYSV-encoded proteins

To gain insights into the functions of the proteins encoded by SiYSV, especially the role of the CP, their subcellular localizations were analyzed. The complete coding sequences of the six proteins encoded by the DNA-A were cloned into pENTR11 followed by recombination into the pK7FWG2 vector, which then expresses the viral protein fused to GFP. The recombinant proteins were transiently expressed in *N. benthamiana* leaves together with AtPIP2A, a protein localized at the plasma membrane, and AtWWP1, a nuclear localized protein. Fluorescent signals were observed at 48 hpi by confocal microscopy.

The subcellular localization of SIYSV-encoded proteins was consistent with previous studies with TYLCV (Li et al., 2020; Wang et al., 2022). Rep was found in the nucleus, but a few granules could be observed in the cytoplasm (Figure 7). TrAP, REn and CP were observed in the nucleus, which correlates to the fact that most of the virus infection cycle, including the steps of DNA replication, transcription of viral genes and encapsidation, occur in this cellular compartment. On the other hand, AC4 was mainly distributed in the plasma membrane, which was previously reported for other geminiviruses (Medina-Puche et al., 2021), and AV2 appears to be located at the nucleus, the membrane and the endoplasmic reticulum. The pattern observed for these two proteins could be explained by their putative roles in viral movement.

7. RNA silencing suppression

Considering the multifunctionality of begomovirus proteins and the ability of these viruses to suppress host antiviral responses, we tested whether the SiYSV-encoded AC4 and TrAP, which have been shown to be silencing suppressors for other begomoviruses, and also the CP, could suppress post-transcriptional RNA silencing in *N. benthamiana*. Firstly, these viral ORFs were cloned into pENTR11 and then recombined into pART27-HA. *N. benthamiana* leaves were co-infiltrated with GFP and each protein of interest, and fluorescence was observed under UV light at 3 and 5 dpi.

While HC-Pro, a known potyviral silencing suppressor, was able to prevent GFP silencing, none of the three SiYSV proteins analyzed showed any silencing activity at either time point (Figure 8). This indicates that SiYSV may not evade plant defense responses by suppression of post-transcriptional RNA silencing (PTGS).

DISCUSSION

In the last decades, a considerable number of new begomoviruses have emerged and many of them have been found in non-cultivated plants. These plants are considered as reservoirs of viral diversity and of new viruses which could spillover and cause diseases in crops (Castillo-Urquiza et al., 2008; Ferro et al., 2017; Fiallo-Olivé et al., 2012; García-Arenal and Zerbini, 2019; Tavares et al., 2003). Recently, our research group described a new begomovirus, Sida yellow spot virus (SiYSV), infecting the non-cultivated plant *S. acuta*. Interestingly, SiYSV encodes a highly divergent CP. Due to the conserved nature of the begomovirus CP, this has raised our interest about the biology of this virus.

We first investigated whether SiYSV could infect other plants besides its natural host. Despite being unable to infect tomato, cotton, *N. tabacum* and the model plant *N. benthamiana*,

SiYSV infected okra plants, demonstrating that it can infect cultivated hosts. Notably, okra belongs to the same plant family than *Sida acuta* (Malvacea). Most plant viruses have a host range restricted to plants of one or two families, although there are some, such as cucumber mosaic virus and tomato spotted wilt virus, that have extremely wide host ranges encompassing more than one thousand plant species in dozens of plant families (Jacquemond, 2012; Parrella et al., 2003). It would be advisable to evaluate additional hosts from other families to unveil the extent of SiYSV's host range, and whether it may be a threat to other economically important crops besides okra.

Since the CP is the only viral protein required for acquisition and transmission by the insect vector (Azzam et al., 1994) and is responsible for vector specificity (Briddon et al., 1990), we hypothesized that SiYSV may not be transmitted by species of the *B. tabaci* complex. Thus, a transmission assay using *B. tabaci* MEAM1 was performed, and indeed SIYSV was not transmitted to any of the inoculated plants.

As mentioned before, *B. tabaci* is a cryptic species complex which currently contains 39 species (Boykin et al., 2017; Vyskočilová et al., 2018). Several of these species are restricted to specific geographical regions, but *B. tabaci* MEAM1 has spread worldwide and is the main species responsible for begomovirus epidemics in most countries (Gilbertson et al., 2015). This polyphagous, highly invasive species was introduced in Brazil in the early 1990's (Lourenção and Nagai, 1994), quickly displaced the indigenous species *B. tabaci* NW1 and NW2 (Ribeiro et al., 1998; Rocha et al., 2011), and is now prevalent in the country (Moraes et al., 2018; Xavier et al., 2021). However, a second invasive species, *B. tabaci* MED, was recently introduced (Barbosa et al., 2015) and since its first report it has quickly spread through the south and southeast regions of Brazil (Bello et al., 2021; De Marchi et al., 2017; Xavier et al., 2021). Thus, it is possible that SiYSV could be transmitted by *B. tabaci* MED.

Gautam et al. (2022) compared the CP amino acid sequences of MED-transmissible and MED-non-transmissible begomoviruses from both the NW and the OW. At residue 93, the MED-non-transmissible viruses have a valine, which is also the case for SiYSV. This suggests that *B. tabaci* MED may not be a vector for SiYSV, although biological experiments need to be perform to confirm this hypothesis.

Begomovirus transmission by *B. tabaci* depends on the interaction of the viral CP and the whitefly GroEL protein. Previous studies have mapped the N-terminal region of the CP as responsible for the interaction with GroEL (Fondong, 2013; Hogenhout et al., 2008). A single amino acid mutation in this region, a substitution of an arginine by leucine at position 19, disrupts the interaction between the tomato yellow leaf curl virus (TYLCV) CP and GroEL, preventing virus transmission by the insect (Yaakov et al., 2011). Our comparison of CP amino acid sequences revealed that SiYSV has an isoleucine at residue 19. Isoleucine is an isomer of leucine with the same physico-chemical properties, which is consistent with the results of our transmission assay indicating that SiYSV is not transmitted by *B. tabaci* MEAM1.

Gottlieb et al. (2010) showed that the TYLCV CP interacts with the GroEL protein synthesized by the secondary endosymbiont *Hamiltonella*, but not with the GroEL produced by *Portiera* and *Rickettsia.* Furthermore, it has been shown that the prevalence of *Hamiltonella* in *B. tabaci* MED populations is associated with increased virus transmission (Bello et al., 2019). Thus, we evaluated whether the SiYSV CP would interact with the GroEL proteins from *Hamiltonella* and *Portiera*. The observed lack of interaction between the SiYSV CP and the GroEL proteins from both endosymbionts is consistent with the inability of *B. tabaci* MEAM1 to transmit SiYSV and also with the sequence analysis indicating that the virus may not be transmitted by *B. tabaci* MED as well.

Although the results of our transmission and BiFC assays as well as sequence analyses all indicate that SiYSV is not transmissible by at least two *B. tabaci* species (MEAM1 and

MED), several studies have indicated that begomovirus transmission may not occur for all whitefly/virus combination (De Marchi et al., 2017; Fiallo-Olivé et al., 2020; Jiu et al., 2006; Li et al., 2010; Liu et al., 2009; Pan et al., 2018). Thus, while the invasive *B. tabaci* MEAM1/MED (which did not co-evolve with the indigenous SiYSV) cannot/may not transmit this virus, it is not unreasonable to assume that indigenous whitefly species such as *B. tabaci* NW1 and NW2 could be capable of transmitting it. Transmission assays using indigenous whitefly species should be carried out before we can definitely establish that SiYSV is not whitefly-transmitted.

Within the family *Geminiviridae*, members of different genera have different types of insect vectors, and this is related with their CP sequences. Vector specificity based on the CP was demonstrated in a classic experiment in which exchange of the CP between a whitefly- and a leafhopper-transmitted virus changed the vector specifity of each virus (Briddon et al., 1990). More recently, phylogeny of the CP was used to correctly infer the possible vector of a new geminivirus (grapevine red blotch virus, a member of the genus *Glabrovirus*) as a treehopper (Bahder et al., 2016). We therefore constructed a phylogenetic tree based on the deduced amino acid sequences of the CP of SiYSV plus 62 geminiviruses classified in all 14 genera of the family. As expected based on its divergent CP, SiYSV did not cluster with members of the genus *Begomovirus*. Its closest relationship was found to be with the three members of the genus *Maldovirus*, which unfortunately have not had their insect vectors identified yet. Together, our results indicate that the search for an insect vector (either an indigenous *B. tabaci* species or a different type of insect) must continue before we can claim that SiYSV does not have a natural vector.

As much as the search for a natural vector must continue, SiYSV can certainly be transmitted by vegetative propagation. Another possibility that should be considered is trans-encapsidation.

Trans-encapsidation is the term used for the complete dependence on the capsid protein of a helper virus to encapsidate the genome of the dependent virus (Singhal et al., 2021). There are several examples of trans-encapsidation occurring in nature. The most well known is trans-encapsidation of the umbravirus groundnut rosette virus (GRV) and its satellite RNA by the luteovirus groundnut rosette assistor virus (GRAV). Umbraviruses do not encode a capsid protein and thus depend on a helper virus for encapsidation and, in the case of the GRV/GRAV system, aphid transmission. The same occurs with begomovirus-associated DNA satellites (alpha- and betasatellites). Alphasatellites encode a replication-associated protein and betasatellites encode a suppressor of gene silencing, however, both depend on a helper virus for encapsidation and whitefly transmission (Zhou, 2013). For trans-encapsidation to occur, both viruses (and satellites) must be in a mixed infection. In fact, mixed infections seem to be the rule rather than the exception for plant viruses (Singhal et al., 2021), and some non-cultivated plants such as *Macroptilium* and *Sida* spp. are reservoirs of virus diversity, co-hosting many different viruses (García-Arenal and Zerbini, 2019; Roye et al., 1997; Tavares et al., 2012). Indeed, the *Sida acuta* plants from which SiYSV was isolated also harbour two additional begomoviruses, Oxalis yellow vein virus (OxYVV) and Sida golden yellow mosaic virus (SiGYMV) (Godinho, 2014). Although SiGYMV also encodes a divergent CP, OxYVV encode a typical begomovirus CP (Xavier, 2015). Thus, it is not unreasonable to assume that SiYSV can be transmitted by trans-encapsidation with the OxYVV CP.

Understanding the function of viral proteins is a key component of a virus biological characterization. Since protein function is intrinsically related to its localization in the cell, we investigated the subcellular localization of SiYSV-encoded proteins. Our results were consistent to what was expected for each protein based on results with other begomoviruses (Hanley-Bowdoin et al., 2013). When DNA viruses infect their host cells, their DNA component (or components) are directed to the nucleus where transcription and replication of

the viral DNA occurs. Rep is essential for the rolling circle replication as it recognize the origin of replication (Fontes et al., 1994), cleaving the DNA strand and providing the free 3'-hydroxyl necessary for the host DNA polymerase to start replicating the viral DNA (Laufs et al., 1995; Orozco et al., 1997). REn is not essential for replication, but it interacts with Rep enhancing the process (Pedersen and Hanley-Bowdoin, 1994; Settlage et al., 1996; Sunter et al., 1990). Therefore, it would be expected that both SiYSV Rep and REn localize at the nucleus, which they did. In turn, TrAP acts as a transcriptional activator of the late genes involved in encapsidation (CP) and movement (NSP) (Saunders and Stanley, 1995; Sunter and Bisaro, 1997). SiYSV TrAP also localized to the nucleus, as expected since this is where transcription occurs.

The begomovirus CP contain a nuclear localization signal (NLS) and it also binds ssDNA, directing the viral DNA to the nucleus (Kunik et al., 1998; Rojas et al., 2001). Also, the nucleus is the site where both newly-synthesized ssDNA and CP accumulate for genome encapsidation (Gutierrez, 1999; Saunders et al., 1991), and is the only site in the cell where viral particles accumulate (Kim et al., 1978). The fact that the SiYSV CP also localizes to the nucleus suggests that, despite its high sequence divergence, it behaves as expected for a typical begomoviral CP.

The SiYSV AC4 protein was found to localize to the plasma membrane (PM), also as reported for other geminiviruses. The C4/AC4 proteins can concentrate at the plasmodesmata (PD), which provides membrane and cytoplasm continuity between cells (Carluccio et al., 2018; Medina-Puche et al., 2021; Rosas-Diaz et al., 2018). The presence of this protein at the PM and plasmodesmata seems to be related to its role in viral movement (Jupin et al., 1994; Teng et al., 2010). The presence of C4/AC4 is also associated to its interaction with receptor-like kinases and interfering with their activities. The TYLCV C4 was shown to interact with the protein BAM1 (BARELY ANY MERISTEM 1), which is also located at the PM and PD, inhibiting

the intercellular movement of silencing signals (siRNAs) usually promoted by BAM1 (Medina-Puche et al., 2021; Rosas-Diaz et al., 2018).

The AV2/V2 protein is supposedly encoded only by OW begomoviruses (Padidam et al., 1996; Rybicki, 1994), however an AV2 ORF is present in the DNA-A of SiYSV (Xavier, 2015). Our subcellular localization assay has shown that the SiYSV AV2 localizes to the PM, the nucleus and apparently also to the endoplasmic reticulum (ER). Previous studies have shown that this protein has similar functions as AC4/C4, being involved in viral movement, symptom development and suppression of host defense responses (Padidam et al., 1996; Rojas et al., 2001). Therefore, it is not unexpected that AV2 displays a similar localization pattern as AC4. Moreover, Moshe et al. (2015) showed that the TYLCV V2 and CP co-localize at the nucleus and that V2 appears to participate in the CP-mediated transport of viral DNA into the nucleus. Concerning the ER localization of AV2, it may also be related with its role in viral movement. Many viral MPs associate with actin filaments and myosin motor proteins to facilitate their movement along the ER to the plasmodesmata (Harries et al., 2010; Moshe et al., 2015; Niehl et al., 2014).

In order to successfully infect its hosts, viruses have to evade their defense responses. The main plant defense response against viruses is RNA silencing. Viruses evolved strategies to overcome this response, encoding suppressors of RNA silencing. Thus, we tested whether SiYSV AC4, TrAP and CP acted as RNA silencing suppressors. AC4 and TrAP were shown to be suppressors of RNA silencing for other begomoviruses (Vanitharani et al., 2004), and we tested the CP due to its divergent nature. Surprisingly, none of them showed any suppression activity, indicating either that SiYSV does not evade plant defense responses by suppression of post-transcriptional RNA silencing (PTGS) or that a different viral protein acts as a suppressor. Moreover, our results do not exclude the possibility that, similarly to other viruses, SiYSV may

overcome RNA silencing by decreasing viral DNA methylation, thus affecting small interfering RNAs (siRNAs) accumulation (Yang et al., 2013).

The present work showed that the SiYSV can infect cultivated hosts and that the SiYSV-encoded proteins display subcellular localizations identical to those of other begomoviruses. The inability of *B. tabaci* MEAM1 to transmit SiYSV may be related to the lack of interaction between its CP and GroEL.

REFERENCES

Ambrozevicius, L.P., Calegario, R.F., Fontes, E.P.B., Carvalho, M.G., Zerbini, F.M., 2002. Genetic diversity of begomoviruses infecting tomato and associated weeds in Southeastern Brazil. Fitopatol Bras 27, 372-377.

Anandalakshmi, R., Pruss, G.J., Ge, X., Marathe, R., Mallory, A.C., Smith, T.H., Vance, V.B., 1998. A viral supressor of gene silencing in plants. Proc Natl Acad Sci USA 95, 13079-13084.

Andrade, E.C., Manhani, G.G., Alfenas, P.F., Calegario, R.F., Fontes, E.P.B., Zerbini, F.M., 2006. *Tomato yellow spot virus*, a tomato-infecting begomovirus from Brazil with a closer relationship to viruses from *Sida* sp., forms pseudorecombinants with begomoviruses from tomato but not from *Sida*. J Gen Virol 87, 3687-3696.

Aragão, F.J.L., Barros, L.M.G., Brasileiro, A.C.M., Ribeiro, S.G., Smith, F.D., Sanford, J.C., Faria, J.C., Rech, E.L., 1996. Inheritance of foreign genes in transgenic bean (*Phaseolus vulgaris* L.) co-transformed via particle bombardment. Theor Appl Genet 93, 142-150.

Azzam, O., Frazer, J., De La Rosa, D., Beaver, J.S., Ahlquist, P.G., Maxwell, D.P., 1994. Whitefly transmission and efficient ssDNA accumulation of bean golden mosaic geminivirus require functional coat protein. Virology 204, 289-296.

Bahder, B.W., Zalom, F.G., Jayanth, M., Sudarshana, M.R., 2016. Phylogeny of geminivirus coat protein sequences and digital PCR aid in identifying *Spissistilus festinus* as a vector of grapevine red blotch-associated virus. Phytopathology 106, 1223-1230.

Barbosa, L.F., Yuki, V.A., Marubayashi, J.M., De Marchi, B.R., Perini, F.L., Pavan, M.A., Barros, D.R., Ghanim, M., Moriones, E., Navas-Castillo, J., Krause-Sakate, R., 2015. First report of *Bemisia tabaci* Mediterranean (Q biotype) species in Brazil. Pest Manag Sci 71, 501-504.

Bello, V.H., da Silva, F.B., Watanabe, L.F.M., Vicentin, E., Muller, C., de Freitas Bueno, R.C.O., Santos, J.C., De Marchi, B.R., Nogueira, A.M., Yuki, V.A., Marubayashi, J.M., Pereira Sartori, M.M., Pavan, M.A., Ghanim, M., Krause-Sakate, R., 2021. Detection of *Bemisia tabaci* Mediterranean cryptic species on soybean in São Paulo and Paraná States (Brazil) and interaction of cowpea mild mottle virus with whiteflies. Plant Pathol 70, 1508-1520.

Bello, V.H., Watanabe, L.F.M., Santos, B.R., Marubayashi, J.M., Yuki, V.A., De Marchi, B.R., Pavan, M.A., Krause-Sakate, R., 2019. Evidence for increased efficiency of virus transmission by populations of Mediterranean species of Bemisia tabaci with high Hamiltonella prevalence. Phytoparasitica 47, 293-300.

Boykin, L.M., Savill, A., De Barro, P., 2017. Updated mtCOI reference dataset for the *Bemisia tabaci* species complex. F1000 Res 6, 1835.

Briddon, R.W., Patil, B.L., Bagewadi, B., Nawaz-ul-Rehman, M.S., Fauquet, C.M., 2010. Distinct evolutionary histories of the DNA-A and DNA-B components of bipartite begomoviruses. BMC Evol Biol 10, 97.

Briddon, R.W., Pinner, M.S., Stanley, J., Markham, P.G., 1990. Geminivirus coat protein gene replacement alters insect specificity. Virology 177, 85-94.

Calil, I.P., Quadros, I.P.S., Araújo, T.C., Duarte, C.E.M., Gouveia-Mageste, B.C., Silva, J.C.F., Brustolini, O.J.B., Teixeira, R.M., Oliveira, C.N., Milagres, R.W.M.M., Martins, G.S., Chory, J., Reis, P.A.B., Machado, J.P.B., Fontes, E.P.B., 2018. A WW domain-containing protein forms immune nuclear bodies against begomoviruses. Mol Plant 11, 1449-1465.

Carluccio, A.V., Prigigallo, M.I., Rosas-Diaz, T., Lozano-Duran, R., Stavolone, L., 2018. S-acylation mediates mungbean yellow mosaic virus AC4 localization to the plasma membrane and in turns gene silencing suppression. PLOS Pathog 14, e1007207.

Castillo-Urquiza, G.P., Beserra Jr., J.E.A., Bruckner, F.P., Lima, A.T.M., Varsani, A., Alfenas-Zerbini, P., Zerbini, F.M., 2008. Six novel begomoviruses infecting tomato and associated weeds in Southeastern Brazil. Arch Virol 153, 1985-1989.

Cutler, S.R., Ehrhardt, D.W., Griffitts, J.S., Somerville, C.R., 2000. Random GFP::cDNA fusions enable visualization of subcellular structures in cells of Arabidopsis at a high frequency. Proc Natl Acad Sci U S A 97, 3718-3723.

Czosnek, H., Hariton-Shalev, A., Sobol, I., Gorovits, R., Ghanim, M., 2017. The incredible journey of begomoviruses in their whitefly vector. Viruses 9, 273.

De Marchi, B.R., Marubayashi, J.M., Favara, G.M., Yuki, V.A., Watanabe, L.F.M., Barbosa, L.F., Pavan, M.A., Krause-Sakate, R., 2017. Comparative transmission of five viruses by *Bemisia tabaci* NW2 and MEAM1. Trop Plant Pathol 42, 495-499.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochem Bull 19, 11-15.

Everett, K.D.E., Thao, M., Horn, M., Dyszynski, G.E., Baumann, P., 2005. Novel chlamydiae in whiteflies and scale insects: endosymbionts '*Candidatus* Fritschea bemisiae' strain Falk and '*Candidatus* Fritschea eriococci' strain Elm. Int J Syst Evol Microbiol 55, 1581-1587.

Ferro, C.G., Silva, J.P., Xavier, C.A.D., Godinho, M.T., Lima, A.T.M., Mar, T.B., Lau, D., Zerbini, F.M., 2017. The ever increasing diversity of begomoviruses infecting non-cultivated hosts: new species from *Sida* spp. and *Leonurus sibiricus*, plus two New World alphasatellites. Ann Appl Biol 170, 204-218.

Fiallo-Olive, E., Lett, J.M., Martin, D.P., Roumagnac, P., Varsani, A., Zerbini, F.M., Navas-Castillo, J., 2021. ICTV Virus Taxonomy Profile: *Geminiviridae* 2021. J Gen Virol 102, 001696.

Fiallo-Olivé, E., Navas-Castillo, J., Moriones, E., Martinez-Zubiaur, Y., 2012. Begomoviruses infecting weeds in Cuba: Increased host range and a novel virus infecting *Sida rhombifolia*. Arch Virol 157, 141-146.

Fiallo-Olivé, E., Pan, L.L., Liu, S.S., Navas-Castillo, J., 2020. Transmission of begomoviruses and other whitefly-borne viruses: Dependence on the vector species. Phytopathology 110, 10-17.

Fondong, V.N., 2013. Geminivirus protein structure and function. Mol Plant Pathol 14, 635-649.

Fontes, E.P.B., Eagle, P.A., Sipe, P.S., Luckow, V.A., Hanley-Bowdoin, L., 1994. Interaction between a geminivirus replication protein and origin DNA is essential for viral replication. J Biol Chem 269, 8459-8465.

García-Arenal, F., Zerbini, F.M., 2019. Life on the edge: geminiviruses at the interface between crops and wild plant hosts. Annu Rev Virol 6, 411-433.

Gautam, S., Mugerwa, H., Buck, J.W., Dutta, B., Coolong, T., Adkins, S., Srinivasan, R., 2022. Differential transmission of Old and New World begomoviruses by Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) cryptic species of *Bemisia tabaci*. Viruses 14, 1104.

Gilbertson, R.L., Batuman, O., Webster, C.G., Adkins, S., 2015. Role of the insect supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. Annu Rev Virol 2, 67-93.

Godinho, M.T., 2014. Coexistência e evolução molecular de populações de begomovírus na planta não-cultivada *Sida acuta*, Dep. de Fitopatologia. Universidade Federal de Viçosa, Viçosa, MG, p. 71.

Gottlieb, Y., Ghanim, M., Chiel, E., Gerling, D., Portnoy, V., Steinberg, S., Tzuri, G., Horowitz, A.R., Belausov, E., Mozes-Daube, N., Kontsedalov, S., Gershon, M., Gal, S., Katzir, N., Zchori-Fein, E., 2006. Identification and localization of a *Rickettsia* sp. in *Bemisia tabaci* (Homoptera: Aleyrodidae). Appl Environ Microbiol 72, 3646-3652.

Gottlieb, Y., Zchori-Fein, E., Mozes-Daube, N., Kontsedalov, S., Skaljac, M., Brumin, M., Sobol, I., Czosnek, H., Vavre, F., Fleury, F., Ghanim, M., 2010. The transmission efficiency of tomato yellow leaf curl virus by the whitefly *Bemisia tabaci* is correlated with the presence of a specific symbiotic bacterium species. J Virol 84, 9310-9317.

Gutierrez, C., 1999. Geminivirus DNA replication. Cell Mol Life Sci 56, 313-329.

Hanley-Bowdoin, L., Bejarano, E.R., Robertson, D., Mansoor, S., 2013. Geminiviruses: Masters at redirecting and reprogramming plant processes. Nat Rev Microbiol 11, 777-788.

Hanley-Bowdoin, L., Settlage, S.B., Orozco, B.M., Nagar, S., Robertson, D., 1999. Geminiviruses: Models for plant DNA replication, transcription, and cell cycle regulation. Crit Rev Plant Sci 18, 71-106.

Harries, P.A., Schoelz, J.E., Nelson, R.S., 2010. Intracellular transport of viruses and their components: Utilizing the cytoskeleton and membrane highways. Mol Plant Microbe Interact 23, 1381-1393.

Harrison, B.D., Swanson, M.M., Fargette, D., 2002. Begomovirus coat protein: serology, variation and functions. Physiol Mol Plant Pathol 60, 257-271.

Heddi, A., Grenier, A.M., Khatchadourian, C., Charles, H., Nardon, P., 1999. Four intracellular genomes direct weevil biology: Nuclear, mitochondrial, principal endosymbiont, and *Wolbachia*. Proc Natl Acad Sci U S A 96, 6814-6819.

Hogenhout, S.A., Ammar, E.D., Whitfield, A.E., Redinbaugh, M.G., 2008. Insect vector interactions with persistently transmitted viruses. Annu Rev Phytopathol 46, 327-359.

Jacquemond, M., 2012. Cucumber mosaic virus. Adv Virus Res 84, 439-504.

Jiu, M., Zhou, X.P., Liu, S.S., 2006. Acquisition and transmission of two begomoviruses by the B and a non-B biotype of Bemisia tabaci from Zhejiang, China. J Phytopathol 154, 587-591.

Johansen, L.K., Carrington, J.C., 2001. Silencing on the spot. Induction and suppression of RNA silencing in the Agrobacterium-mediated transient expression system. Plant Physiol 126, 930-938.

Jupin, I., Kouchkovsky, F., Jouanneau, F., Gronenborn, B., 1994. Movement of tomato yellow leaf curl geminivirus (TYLCV): Involvement of the protein encoded by ORF C4. Virology 204, 82-90.

Katoh, K., Misawa, K., Kei-ichi, K., Miyata, T., 2012. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30, 3059-3066.

Kim, K.S., Shock, T.L., Goodman, R.M., 1978. Infection of *Phaseolus vulgaris* by bean golden mosaic virus: ultrastructural aspects. Virology 89, 22-33.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35, 1547-1549.

Kunik, T., Palanichelvam, K., Czosnek, H., Citovsky, V., Gafni, Y., 1998. Nuclear import of the capsid protein of tomato yellow leaf curl virus (TYLCV) in plant and insect cells. Plant J 13, 393-399.

Laufs, J., Traut, W., Heyraud, F., Matzeit, G., Rogers, S.G., Schell, J., Gronenborn, B., 1995. *In vitro* cleavage and joining at the viral origin of replication by the replication initiator protein of tomato yellow leaf curl virus. Proc Natl Acad Sci USA 92, 3879-3883.

Legg, J., Fauquet, C., 2004. Cassava mosaic geminiviruses in Africa. Plant Mol Biol 56, 585-599.

Li, F., Xu, X., Huang, C., Gu, Z., Cao, L., Hu, T., Ding, M., Li, Z., Zhou, X., 2015. The AC5 protein encoded by Mungbean yellow mosaic India virus is a pathogenicity determinant that suppresses RNA silencing-based antiviral defenses. New Phytol 208, 555-569.

Li, H., Li, F., Zhang, M., Gong, P., Zhou, X., 2020. Dynamic subcellular localization, accumulation, and interactions of proteins from tomato yellow leaf curl China virus and Its associated betasatellite. Front Plant Sci 11, 840.

Li, M., Hu, J.A., Xu, F.C., Liu, S.S., 2010. Transmission of tomato yellow leaf curl virus by two invasive biotypes and a Chinese indigenous biotype of the whitefly *Bemisia tabaci*. Int J Pest Managem 56, 275-280.

Liu, J., Zhao, H., Jiang, K., Zhou, X.P., Liu, S.S., 2009. Differential indirect effects of two plant viruses on an invasive and an indigenous whitefly vector: implications for competitive displacement. Ann Appl Biol 155, 439-448.

Llave, C., Kasschau, K.D., Carrington, J.C., 2000. Virus-enconded suppressor of posttranscriptional gene silencing targets a maintenance step in the silencing pathway. Proc Natl Acad Sci USA 97, 13401-13406.

Lourenção, A.L., Nagai, H., 1994. Surtos populacionais de *Bemisia tabaci* no Estado de São Paulo. Bragantia 53, 53-59.

Martin, K., Kopperud, K., Chakrabarty, R., Banerjee, R., Brooks, R., Goodin, M.M., 2009. Transient expression in *Nicotiana benthamiana* fluorescent marker lines provides enhanced definition of protein localization, movement and interactions *in planta*. Plant J 59, 150-162.

Medina-Puche, L., Orilio, A.F., Zerbini, F.M., Lozano-Duran, R., 2021. Small but mighty: Functional landscape of the versatile geminivirus-encoded C4 protein. PLOS Pathog 17, e1009915.

Moraes, L.A., Muller, C., Bueno, R., Santos, A., Bello, V.H., De Marchi, B.R., Watanabe, L.F.M., Marubayashi, J.M., Santos, B.R., Yuki, V.A., Takada, H.M., Barros, D.R., Neves, C.G., Silva, F.N., Goncalves, M.J., Ghanim, M., Boykin, L., Pavan, M.A., Krause-Sakate, R., 2018. Distribution and phylogenetics of whiteflies and their endosymbiont relationships after the Mediterranean species invasion in Brazil. Sci Rep 8, 14589.

Morin, S., Ghanim, M., Zeidan, M., Czosnek, H., Verbeek, M., van den Heuvel, J.F., 1999. A GroEL homologue from endosymbiotic bacteria of the whitefly *Bemisia tabaci* is implicated in the circulative transmission of tomato yellow leaf curl virus. Virology 256, 75-84.

Moriones, E., Navas-Castillo, J., 2000. *Tomato yellow leaf curl virus*, an emerging virus complex causing epidemics worldwide. Virus Res 71, 123-134.

Moshe, A., Belausov, E., Niehl, A., Heinlein, M., Czosnek, H., Gorovits, R., 2015. The Tomato yellow leaf curl virus V2 protein forms aggregates depending on the cytoskeleton integrity and binds viral genomic DNA. Sci Rep 5, 9967.

Muyzer, G., Hottentrager, S., Teske, A., Wawer, C., 1996. Denaturing gradient gel electrophoresis of PCR amplified 16s rDNA – A new molecular approach to analyze the genetic diversity of mixed microbial communities, in: Akkermans, A.D.L., van Elsas, J.D., Bruijn, F.J. (Eds.), Molecular Microbial Ecology Manual. Kluwer Academic Publishers, Dordrecht.

Navas-Castillo, J., Fiallo-Olivé, E., Sánchez-Campos, S., 2011. Emerging virus diseases transmitted by whiteflies. Annu Rev Phytopathol 49, 219-248.

Niehl, A., Pasquier, A., Ferriol, I., Mély, Y., Heinlein, M., 2014. Comparison of the oilseed rape mosaic virus and tobacco mosaic virus movement proteins (MP) reveals common and dissimilar MP functions for tobamovirus spread. Virology 456-457, 43-54.

Oliveira, P.G.S., 2021. Obtenção e caracterização de clones infecciosos de um begomovírus isolado de *Sida acuta*, Dep. de Bioquímica e Biologia Molecular. Universidade Federal de Viçosa, Viçosa, MG.

Orozco, B.M., Miller, A.B., Settlage, S.B., Hanley-Bowdoin, L., 1997. Functional domains of a geminivirus replication protein. J Biol Chem 272, 9840-9846.

Padidam, M., Beachy, R.N., Fauquet, C.M., 1996. The role of AV2 ("precoat") and coat protein in viral replication and movement in tomato leaf curl geminivirus. Virology 224, 390-404.

Pan, L., Chen, Q., Guo, T., Wang, X., Li, P., Wang, X., Liu, S., 2018. Differential efficiency of a begomovirus to cross the midgut of different species of whiteflies results in variation of virus transmission by the vectors. Sci China Life Sci 61, 1254-1265.

Parrella, G., Gognalons, P., Gebre-Selassie, K., Vovlas, C., Marchoux, G., 2003. An update of the host range of tomato spotted wilt virus. J Plant Pathol 85, 227-264.

Pedersen, T.J., Hanley-Bowdoin, 1994. Molecular characterization of the AL3 protein encoded by a bipartite geminivirus. Virology 202, 1070-1075.

Ribeiro, S.G., Ávila, A.C., Bezerra, I.C., Fernandes, J.J., Faria, J.C., Lima, M.F., Gilbertson, R.L., Zambolim, E.M., Zerbini, F.M., 1998. Widespread occurrence of tomato geminiviruses in Brazil, associated with the new biotype of the whitefly vector. Plant Dis 82, 830.

Rocha, K.C.G., Marubayashi, J.M., Navas-Castillo, J., Yuki, V.A., Wilcken, C.F., Pavan, M.A., Krause-Sakate, R., 2011. Only the B biotype of *Bemisia tabaci* is present on vegetables in Sao Paulo State, Brazil. Sci Agr 68, 120-123.

Rojas, M.R., Gilbertson, R.L., Russell, D.R., Maxwell, D.P., 1993. Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. Plant Dis 77, 340-347.

Rojas, M.R., Jiang, H., Salati, R., Xoconostle-Cazares, B., Sudarshana, M.R., Lucas, W.J., Gilbertson, R.L., 2001. Functional analysis of proteins involved in movement of the monopartite begomovirus, tomato yellow leaf curl virus. Virology 291, 110-125.

Rojas, M.R., Macedo, M.A., Maliano, M.R., Soto-Aguilar, M., Souza, J.O., Briddon, R.W., Kenyon, L.A., Rivera-Bustamante, R.F., Zerbini, F.M., Adkins, S., Legg, J.P., Kvarnheden, A., Wintermantel, W.M., Sudarshana, M.R., Peterschmitt, M., Lapidot, M., Martin, D.P., Moriones, E., Inoue-Nagata, A.K., Gilbertson, R.L., 2018. World management of geminiviruses. Annu Rev Phytopathol 56, 637-677.

Rosas-Diaz, T., Zhang, D., Fan, P., Wang, L., Ding, X., Jiang, Y., Jimenez-Gongora, T., Medina-Puche, L., Zhao, X., Feng, Z., Zhang, G., Liu, X., Bejarano, E.R., Tan, L., Zhang, H., Zhu, J.K., Xing, W., Faulkner, C., Nagawa, S., Lozano-Duran, R., 2018. A virus-targeted plant receptor-like kinase promotes cell-to-cell spread of RNAi. Proc Natl Acad Sci U S A 115, 1388-1393.

Roumagnac, P., Lett, J.M., Fiallo-Olive, E., Navas-Castillo, J., Zerbini, F.M., Martin, D.P., Varsani, A., 2021. Establishment of five new genera in the family *Geminiviridae*: *Citlodavirus*, *Maldovirus*, *Mulcrilevirus*, *Opunvirus*, and *Topilevirus*. Arch Virol 167, 695-710.

Roye, M.E., McLaughlin, W.A., Nakhla, M.K., Maxwell, D.P., 1997. Genetic diversity among geminiviruses associated with the weed species *Sida* spp., *Macroptilium lathyroides*, and *Wissadula amplissima* from Jamaica. Plant Dis 81, 1251-1258.

Rybicki, E.P., 1994. A phylogenetic and evolutionary justification for three genera of *Geminiviridae*. Arch Virol 139, 49-77.

Saunders, K., Lucy, A., Stanley, J., 1991. DNA forms of the geminivirus African cassava mosaic virus are consistent with a rolling circle mechanism of replication. Nucleic Acids Res 19, 2325-2330.

Saunders, K., Stanley, J., 1995. Complementation of African cassava mosaic virus AC2 gene function in a mixed bipartite geminivirus infection. J Gen Virol 76, 2287-2292.

Settlage, S.B., Miller, A.B., Hanley-Bowdoin, L., 1996. Interactions between geminivirus replication proteins. J Virol 70, 6790-6795.

Singhal, P., Nabi, S.U., Yadav, M.K., Dubey, A., 2021. Mixed infection of plant viruses: Diagnostics, interactions and impact on host. J Plant Dis Protect 128, 353-368.

Stamatakis, A., 2006. RAxML-VI-HPC: Maximum Likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinf Applic Note 22, 2688-2690.

Sunter, G., Bisaro, D.M., 1997. Regulation of a geminivirus coat protein promoter by AL2 protein (TrAP): Evidence for activation and derepression mechanisms. Virology 232, 269-280.

Sunter, G., Hartitz, M.D., Hormuzdi, S.G., Brough, C.L., Bisaro, D.M., 1990. Genetic analysis of tomato golden mosaic virus: ORF AL2 is required for coat protein accumulation while ORF AL3 is necessary for eficient DNA replication. Virology 179, 69-77.

Tavares, E.T., Tatagiba, J.S., Martins, N.F., Silva, F.R., Marinho, V.L.A., Ventura, J.A., Zambolim, E.M., M.T., S.J., 2003. Deciphering the genome of the virus causing "Papaya Sticky Disease". Fitopatol Bras 28, S253-254.

Tavares, S.S., Ramos-Sobrinho, R., Gonzalez-Aguilera, J., Lima, G.S.A., Assunção, I.P., Zerbini, F.M., 2012. Further molecular characterization of weed-associated begomoviruses in Brazil with an emphasis on *Sida* spp. Planta Dan 30, 305-315.

Teng, K.L., Chen, H., Lai, J.B., Zhang, Z.H., Fang, Y.Y., Xia, R., Zhou, X.P., Guo, H.S., Xie, Q., 2010. Involvement of C4 protein of beet severe curly top virus (family *Geminiviridae*) in virus movement. PLOS ONE 5, e11280.

Thao, M.L., Baumann, P., 2004. Evolutionary relationships of primary prokaryotic endosymbionts of whiteflies and their hosts. Appl Environ Microbiol 70, 3401-3406.

Vanitharani, R., Chellappan, P., Pita, J.S., Fauquet, C.M., 2004. Differential roles of AC2 and AC4 of cassava geminiviruses in mediating synergism and suppression of posttranscriptional gene silencing. J Virol 78, 9487-9498.

Vyskočilová, S., Tay, W.T., van Brunschot, S., Seal, S., Colvin, J., 2018. An integrative approach to discovering cryptic species within the *Bemisia tabaci* whitefly species complex. Sci Rep 8, 10886.

Walsh, P.S., Metzger, D.A., Higuchi, R., 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10, 506-513.

Wang, L., Tan, H., Medina-Puche, L., Wu, M., Garnelo Gomez, B., Gao, M., Shi, C., Jimenez-Gongora, T., Fan, P., Ding, X., Zhang, D., Ding, Y., Rosas-Díaz, T., Liu, Y., Aguilar, E., Fu, X., Lozano-Durán, R., 2022. Combinatorial interactions between viral proteins expand the potential functional landscape of the tomato yellow leaf curl virus proteome. PLOS Pathog 18, e1010909.

Weeks, A.R., Velten, R., Stouthamer, R., 2003. Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthropods. Proc Biol Sci 270, 1857-1865.

Wise, A.A., Liu, Z., Binns, A.N., 2006. Three methods for the introduction of foreign DNA into Agrobacterium. Methods Mol Biol 343, 43-53.

Xavier, C.A.D., 2015. Species diversity and genetic variability of bipartite begomoviruses in the New World, Dep. de Fitopatologia. Universidade Federal de Viçosa, Viçosa, MG, p. 106.

Xavier, C.A.D., Nogueira, A.M., Bello, V.H., Watanabe, L.F.M., Barbosa, T.M.C., Alves-Júnior, M., Barbosa, L., Beserra-Júnior, J.E.A., Boari, A., Calegario, R., Gorayeb, E.S., Honorato-Júnior, J., Koch, G., Lima, G.S.A., Lopes, C., Mello, R.N., Pantoja, K., Silva, F.N., Ramos-Sobrinho, R., Santana, E.N., Silva, J.W.P., Krause-Sakate, R., Zerbini, F.M., 2021. Assessing the diversity of whiteflies infesting cassava in Brazil. PeerJ 9, e11741.

Yaakov, N., Levy, Y., Belausov, E., Gaba, V., Lapidot, M., Gafni, Y., 2011. Effect of a single amino acid substitution in the NLS domain of tomato yellow leaf curl virus-Israel (TYLCV-IL) capsid protein (CP) on its activity and on the virus life cycle. Virus Res 158, 8-11.

Yang, L.P., Fang, Y.Y., An, C.P., Dong, L., Zhang, Z.H., Chen, H., Xie, Q., Guo, H.S., 2013. C2-mediated decrease in DNA methylation, accumulation of siRNAs, and increase in expression for genes involved in defense pathways in plants infected with beet severe curly top virus. Plant J 73, 910-917.

Zchori-Fein, E., Brown, J.K., 2002. Diversity of prokaryotes associated with *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Ann Entomol Soc Am 95, 711-718.

Zhou, X., 2013. Advances in understanding begomovirus satellites. Annu Rev Phytopathol 51, 357-381.

Table 1. Primers used for PCR amplification of viral and endosymbiont genes and cloning into the pENTR11 vector. Restriction sites present in some of the primers are highlighted in bold.

Primer name	Primer sequence	Restriction enzyme
SiY-2F	5'-GAGTTATCCCTATGTCCCCC-3'	-
SiY2-R	5'-GAGAATGGCGTCTATACCTGG-3'	-
Rep FW	5'-GGG**GGATCC**GGATGGGTACAAGTTACATT-3'	BamHI
Rep RV	5'-TTT**CTCGAG**AACGGCGAATTCCTCTCTTC-3'	Xho I
CP FW	5'-GGG**GGATCC**ATATGGATTACAGGAAG-3'	BamHI
CP RV	5'-GGG**CTCGAG**AATGAATATCCAGATACG-3'	Xho I
Trap FW	5'-GGG**GGATCC**GGATGCTAAATTCATCT-3'	BamHI
Trap RV	5'-GGG**GATATC**AAATTAAATAAGTCGTT-3'	EcoRV
C4 FW	5'-GAG**GGATCC**AAATGAAAATGGGGAGC-3'	BamHI
C4 RV	5'-GGA**CTCGAG**AAGTATATTGAGGGCCT-3'	Xho I
Ren FW	5'-AAA**GGATCC**AAATGGATTCACGCACAGGG-3'	BamHI
Ren RV	5'-GGGG**CTCGAG**GGATAAAGCTTGAATTTTAT-3'	Xho I
AV2-like FW	5'-TTA**GGATCC**AAATGAGAGTGCGCGTT-3'	BamHI
AV2-like RV	5'-GGG**CTCGAG**AATTCGTCAATCGAAAC-3'	Xho I
GroEL Portiera FW	5'-GGG**GGATCC**AAATGGCAGCAAAACA-3'	BamHI
GroEL Portiera RV	5'-GGG**CTCGAG**GGAGATCTCATACCATT-3'	Xho I
GroEL Hamiltonella FW	5'-GGG**GGATCC**GAATGGCAGCTAAAGACTTA-3'	BamHI
GroEL Hamiltonella RV	5'-GGG**CTCGAG**GACATCATACCATTCATTCC-3'	Xho I

Table 2. Primers used for identification of whitefly endosymbionts.

Endosymbiont	Primer name	Primer sequence	Reference
Portiera	Port16S (FW)	5'-CGCCCGCCGCGCCCCGCGCCCGTCCC GCCGCCCCCGCCCG-3'	Muyzer et al. (1996)
	Port16S (RV)	5'-CCGTCAATTCMTTTGAGTTT-3'	
Rickttesia	Rick16S (FW)	5'-GCTCAGAACGAACGCTATC-3'	Gottlieb et al. (2006)
	Rick16S (RV)	5'-GAAGGAAAGCATCTCTGC-3'	
Hamiltonella	Ham16S (FW)	5'-TGAGTAAAGTCTGGAATCTGG-3'	Zchori-Fein and Brown (2002)
	Ham16S (RV)	5'-AGTTCAAGACCGCAACCTC-3'	
Wolbachia	Wol16S (FW)	5'-CGGGGGAAAAATTTATTGCT-3'	Heddi et al. (1999)
	Wol16S (RV)	5'-AGCTGTAATACAGAAAGTAAA-3'	
Arsenophonus	Ars23S (FW)	5'-CGTTTGATGAATTCATAGTCAAA-3'	Thao and Baumann (2004)
	Ars23S (RV)	5'-GGTCCTCCAGTTAGTGTTACCCAAC-3'	
Cardinium	Cardi16S (FW)	5'-GCGGTGTAAAATGAGCGTG-3'	Weeks et al. (2003)
	Cardi16S (RV)	5'-ACCTMTTCTTAACTCAAGCCT-3'	
Fritchea	Frit23S (FW)	5'-GATGCCTTGGCATTGATAGGCGATG AAGGA-3'	Everett et al. (2005)
	Frit23S (RV)	5'-TGGCTCATCATGCAAAAGGCA-3'	

Table 3 - Geminivirus sequences retrieved from GenBank.

Genus	***Species***	**Acronym**	**Access number**	**Vector**	**Origin**
Becurtovirus	*Beet curly top Iran virus*	BCTIV	EU273818	*Circulifer haematoceps* (Cicadellidae)	OW
	Spinach curly top Arizona virus	SCTAV	HQ443515	*C. haematoceps*	OW
	Exomis microphylla latent virus	EMLV	NC_037065	*C. haematoceps*	OW
Begomovirus	*Abutilon mosaic Brazil virus*	AbMBV	NC_016574	*Bemisia tabaci* (Aleyrodidae)	NW
	Abutilon mosaic virus	AbMV	NC_001928	*B. tabaci*	NW
	Bean golden mosaic virus	BGMV	NC_004042	*B. tabaci*	NW
	Blainvillea yellow spot virus	BlYSV	NC_010837	*B. tabaci*	NW
	Cotton leaf crumple virus	CLCrV	NC_004580	*B. tabaci*	NW
	Euphorbia mosaic virus	EuMV	NC_008304	*B. tabaci*	NW
	Euphorbia yellow mosaic virus	EuYMV	NC_012553	*B. tabaci*	NW
	Oxalis yellow vein virus	OxYVV	NC_026253	*B. tabaci*	NW
	Passionfruit severe leaf distortion virus	PSLDV	NC_012786	*B. tabaci*	NW
	Sida golden mosaic virus	SiGMV	NC_002046	*B. tabaci*	NW
	Sida yellow leaf curl virus	SiYLCV	NC_038461	*B. tabaci*	NW
	Sida yellow mosaic virus	SiYMV	NC_004639	*B. tabaci*	NW
	Sweet potato leaf curl virus	SPLCV	NC_004650	*B. tabaci*	NW
	Tomato common mosaic virus	ToCmMV	NC_010835	*B. tabaci*	NW
	Tomato rugose mosaic virus	ToRMV	NC_002555	*B. tabaci*	NW
	Tomato severe rugose virus	ToSRV	NC_009607	*B. tabaci*	NW
	Tomato yellow spot virus	ToYSV	NC_007726	*B. tabaci*	NW
	African cassava mosaic virus	ACMV	FN668378	*B. tabaci*	OW
	Ageratum yellow vein virus	AYVV	NC_004090	*B. tabaci*	OW
	Corchorus golden mosaic virus	CoGMV	NC_009644	*B. tabaci*	OW
	East African cassava mosaic virus	EACMV	NC_004674	*B. tabaci*	OW
	Indian cassava mosaic virus	ICMV	NC_001932	*B. tabaci*	OW
	Ludwigia yellow vein virus	LuYVV	NC_007210	*B. tabaci*	OW
	Sauropus leaf curl virus	SaLCV	NC_038455	*B. tabaci*	OW
	Tomato yellow leaf curl virus	TYLCV	NC_004005	*B. tabaci*	OW
	Watermelon chlorotic stunt virus	WmCSV	NC_003708	*B. tabaci*	OW
Capulavirus	*Alfalfa leaf curl virus*	ALCV	KP732474	*Aphis craccivora* (Aphididae)	OW
	French bean severe leaf curl virus	FbSLSV	JX094280	Not identified	OW
	Euphorbia caput-medusae latent virus	EcmLV	HF921459	*A. craccivora*	OW
	Plantago lanceolata latent virus	PlLV	KT214389	*Dysaphis plantaginea* (Aphididae)	OW
Citlodavirus	*Camellia chlorotic dwarf-associated virus*	CaCDaV	MG452759	Not identified	OW
	Citrus chlorotic dwarf associated virus	CCDaV	MT683770	*Parabemisia myricae* (Aleyrodidae)	OW
	Paper mulberry leaf curl virus 2	PMLCV2	MN595128	Not identified	OW
	Passion fruit chlorotic mottle virus	PFCMV	NC_040706	Not identified	OW
Curtovirus	*Beet curly top virus*	BCTV	NC_001412	Leafhoppers (Cicadellidae)	OW
	Horseradish curly top virus	HCTV	NC_002543	Leafhoppers (Cicadellidae)	OW
	Spinach severe curly top virus	SpSCTV	NC_014631	Leafhoppers (Cicadellidae)	OW

Table 3 (cont.)

Eragrovirus	*Eragrostis curvula streak virus*	ECSV	NC_012664	Not identified	OW
Grablovirus	*Grapevine red blotch virus*	GRBV	NC_022002	*Spissistilus festinus* (Membracidae)	NW
	Prunus latent virus	PrLV	NC_043533	*S. festinus*	NW
	Wild vitis latent virus	WvLV	NC_035480	*S. festinus*	NW
Maldovirus	*Apple geminivirus 1*	AG1	KM386645	Not identified	OW
	Grapevine geminivirus A	GGA	KX570607	Not identified	OW
	Juncus maritimus geminivirus 1	JMG1	MG001958	Not identified	OW
Mastrevirus	*Chickpea chlorotic dwarf virus*	CpCDV	NC_011058	Leafhoppers (Cicadellidae)	OW
	Maize streak virus	MSV	NC_001346	Leafhoppers (Cicadellidae)	OW
	Maize striate mosaic virus	MSMV	NC_040541	Leafhoppers (Cicadellidae)	OW
	Oat dwarf virus	ODV	NC_010799	Leafhoppers (Cicadellidae)	OW
	Sugarcane chlorotic streak virus	SCSV	NC_032004	Leafhoppers (Cicadellidae)	OW
	Tobacco yellow dwarf virus	TYDV	NC_003822	Leafhoppers (Cicadellidae)	OW
Mulcrilevirus	*Mulberry crinkle leaf virus*	MCLV	MW741881	*Tautoneura mori* (Cicadellidae)	OW
	Paper mulberry leaf curl virus 1	PMLCV1	MN595125	Not identified	OW
Opunvirus	*Opuntia virus 1*	OV1	NC_055584	Not identified	NW
Topilevirus	*Tomato apical leaf curl virus*	TALCV	MG491195	Not identified	NW
	Tomato geminivirus 1	TG1	MF072689	Not identified	NW
Topocuvirus	*Tomato pseudo-curly top virus*	TPCTV	NC_003825	*Micrutalis malleifera* (Membracidae)	NW
Turncurtovirus	*Turnip curly top virus*	TCTV	NC_014324	*Circulifer haematoceps* (Cicadellidae)	OW
	Turnip leaf roll virus	TuLCV	NC_029117	*C. haematoceps*	OW
	Sesame curly top virus	SeCTV	MT041697	*C. haematoceps*	OW

Table 4. Results of biolistic inoculation of Sida yellow spot virus (SiYSV) in plants of the families Solanaceae and Malvaceae.

Family	Species/cultivar	SiYSV		ToYSV		Mock	
		Inf./Inoc.[1]	Sint.[2]	Inf./Inoc.	Sint.	Inf./Inoc.	Sint.
Solanaceae	*Solanum lycopersicum* 'Santa Clara'	0/19	-	9/10	ym, ld,lr	0/4	-
	Nicotiana benthamiana	0/17	-	6/8	ym, ld	0/4	-
	Nicotiana tabacum 'TNN'	0/17	-	5/10	As	0/2	-
Malvaceae	*Abelmoschus esculentum*	7/17	ym, ld	0/9	-	0/4	-
	Gossypium hirsutum	0/20	-	-	-	0/4	-
	Sida acuta	6/16	ym	2/7	As	0/2	-

[1] Number of infected plants/number of inoculated plants.
[2] Symptoms on infected plants at 28 dpi: as, asymptomatic; ld, leaf deformation; lr, leaf rugosity; ym, yellow mosaic.

Table 5. Results of whitefly (*Bemisia tabaci* MEAM1) inoculation of SiYSV and OxYVV in *Sida acuta* plants.

SiYSV		**OxYVV**		**Mock**	
Inf./Inoc.[1]	Sint.[2]	Inf./Inoc.	Sint.	Inf./Inoc.	Sint.
0/65	-	5/11	vy, ym	0/6	-

[1] Number of infected plants/number of inoculated plants.
[2] Symptoms on infected plants at 48 dpi: vy, vein yellowing; ym, yellow mosaic.

Figure legends

Figure 1. Schematic representation of the whitefly transmission assay. (A) Aviruliferous whiteflies were maintained in *Sida acuta* plants infected with Oxalis yellow vein virus (OxYVV) and Sida yellow spot virus (SiYSV) for an acquisition-access period (AAP) of 72 h and kept at insect-free cages. After this period, (B) the whiteflies were moved to healthy *S. acuta* plants for an inoculation-access period (IAP) of 72 h. Plants were transferred to a greenhouse and symptom development was monitored until 40 dpi.

Figure 2. Symptoms induced in *Sida acuta* and *Abelmoschus esculentus* (okra) plants, inoculated by biolistic with infectious clones of Sida yellow spot virus (SiYSV). Images were obtained at 28 days post-inoculation.

Figure 3. *Sida acuta* plants inoculated by *B. tabaci* MEAM1 in the transmission assay. Whiteflies fed on plants infected with Oxalis yellow vein virus (OxYVV) or Sida yellow spot virus (SiYSV) for a period of 72 h and then were transferred to healthy plants for an inoculation-access period (IAP) of 72 h. Whiteflies that fed on mock-inoculated plants were also transferred to healthy plants and these were used as negative control. The development of symptoms was observed until 40 dpi. Plants inoculated with OxYVV showed characteristic symptoms of yellow mosaic and vein yellowing while SiYSV-inoculated plants did not show any symptoms.

Figure 4. BiFC assay showing no interaction between the Sida yellow spot virus (SiYSV) CP and the GroEL proteins of the endosymbionts *Portiera* (PGroEL) and *Hamiltonella* (HGroEL). *N. benthamiana* leaves were infiltrated with constructs expressing CP and GroEL fused to the

YFP N-terminus (nYFP) or C-terminus (cYFP) and then observed in a laser scanning confocal microscope. Images were taken at 48 hours post-inoculation. Scale bar: 20 μm.

Figure 5. Comparison of capsid protein amino acid sequences of Sida yellow spot vrius (SiYSV), three maldoviruses and 14 begomoviruses. At amino acid residue 93 (highlighted in yellow), SiYSV and *B. tabaci* MED-nontransmissible viruses have a valine (V), whereas *B. tabaci* MED-transmissible have an isoleucine (I). At residue 19, where the mutation R19L was reported to disrupt the interaction between CP and GroEL (highlighted in green), SiYSV has an isoleucine. The underlined regions indicate conserved domains of OW and NW begomoviruses.

Figure 6. Maximum-likelihood phylogenetic tree based on the deduced amino acid sequences of 62 geminiviruses including Sida yellow spot virus (SiYSV). SiYSV did not group with members of the genus *Begomovirus*. Instead, it grouped with members of the genus *Maldovirus*.

Figure 7. Subcellular localization of Sida yellow spot virus (SiYSV)-encoded proteins fused to GFP at their C-terminus. *N. bethamiana* leaves were co-infiltrated with Rep-GFP, REn-GFP, TrAP-GFP, AC4-GFP, CP-GFP or AV2-GFP, and the nuclear and membrane markers AtWWP1 and AtPIP2A, respectively, fused to mCherry, and then observed in a laser scanning confocal microscope. As a negative control, leaves were infiltrated with empty vector. Images were taken at 48 hours post-inoculation. Scale bar: 50 μm.

Figure 8. RNA silencing suppression assay. *N. bethamiana* leaves were co-infiltrated with binary vectors expressing HC-Pro and GFP in one half, and in the other half binary vectors expressing GFP and Sida yellow spot vrius (SiYSV) CP or AC4 or TrAP or the empty vector

(negative control). These leaves were observed under UV light at 3 and at 5 days poist-inoculation for detection of GFP fluorescence, indicative of RNA silencing suppression. No SiYSV proteins tested showed any sign of post-transcription RNA silencing suppression.

Figure 1

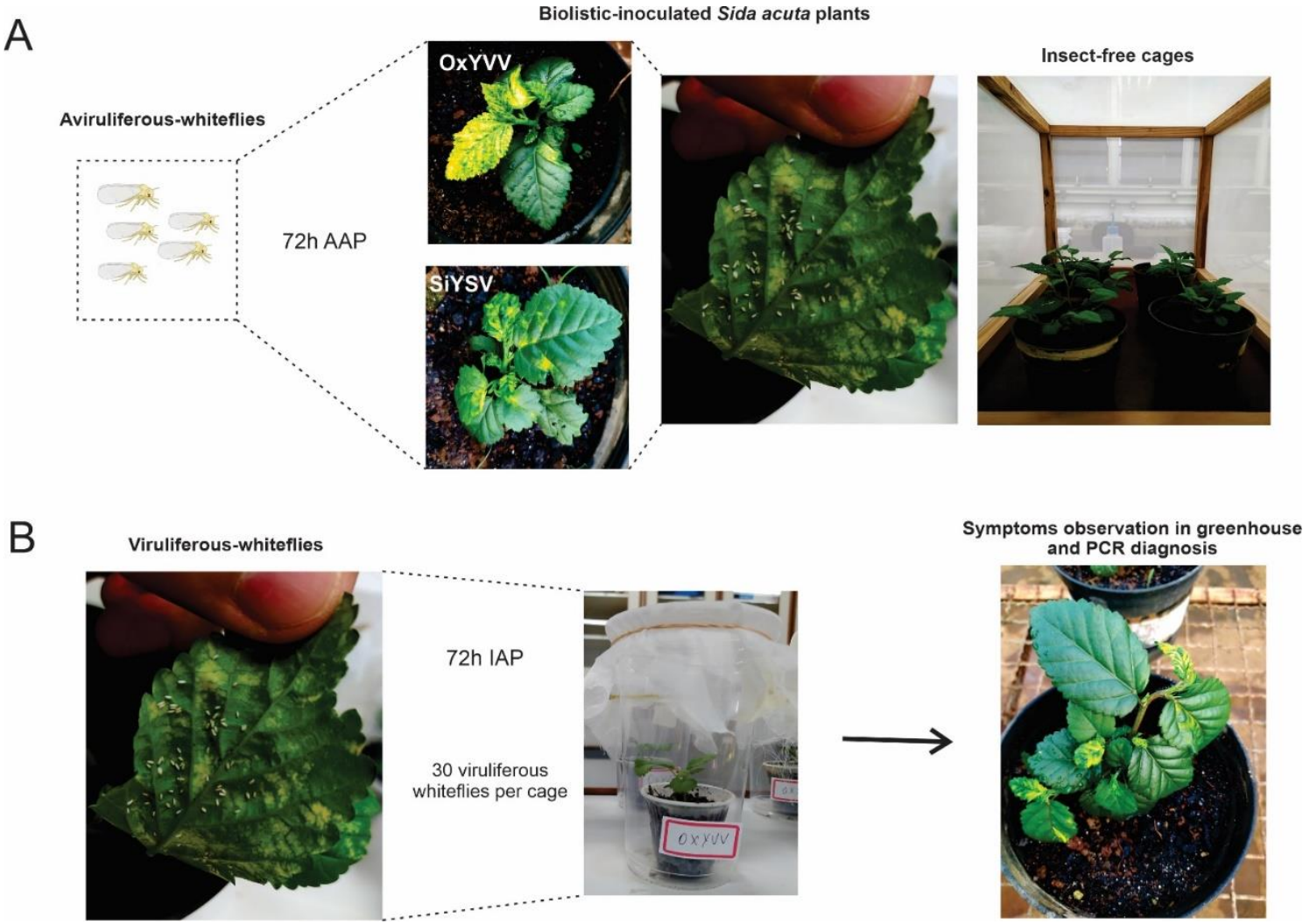

A
Biolistic-inoculated *Sida acuta* plants
Aviruliferous-whiteflies
72h AAP
OxYVV
SiYSV
Insect-free cages
B
Viruliferous-whiteflies
72h IAP
30 viruliferous whiteflies per cage
OxYVV
Symptoms observation in greenhouse and PCR diagnosis

Figure 2

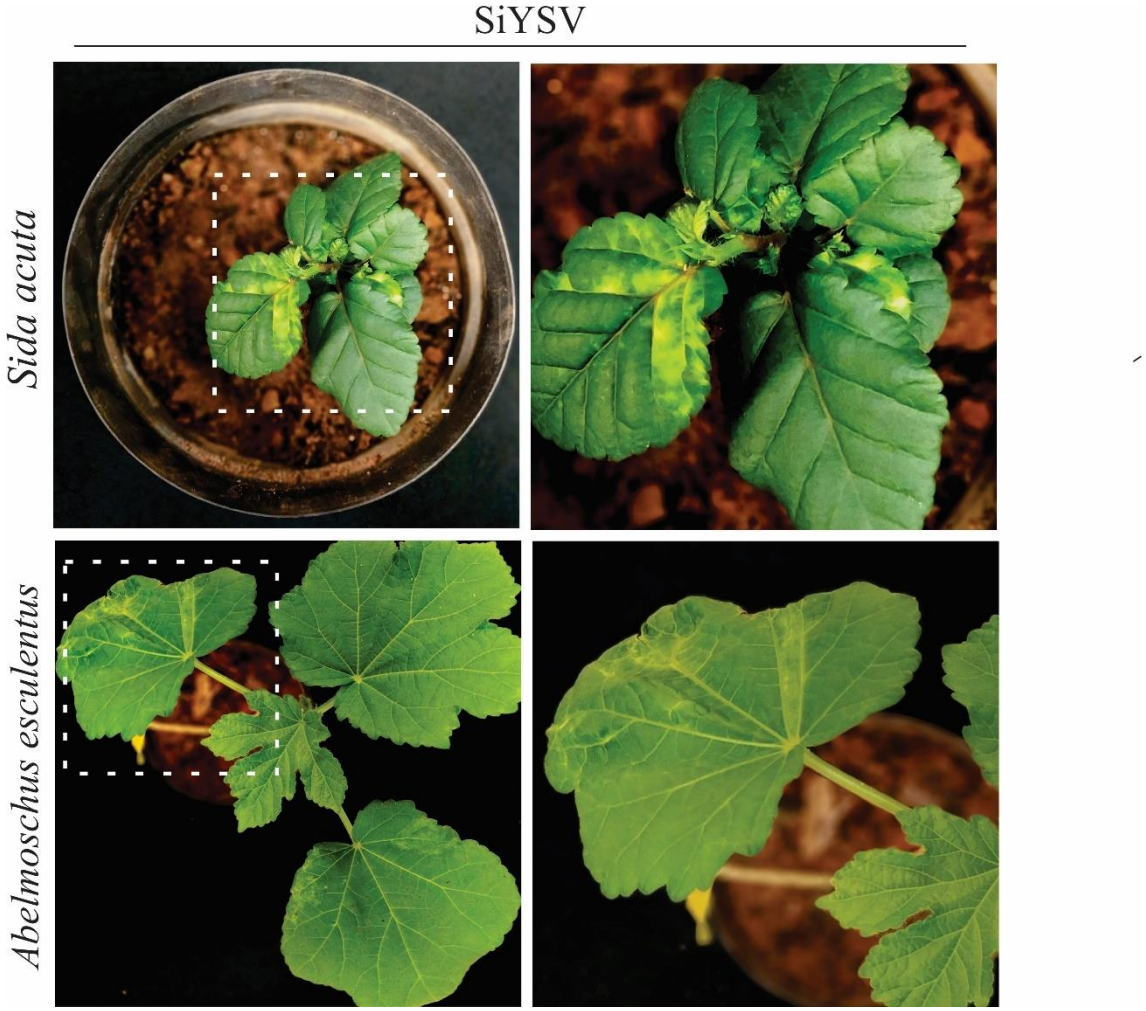


Figure 3

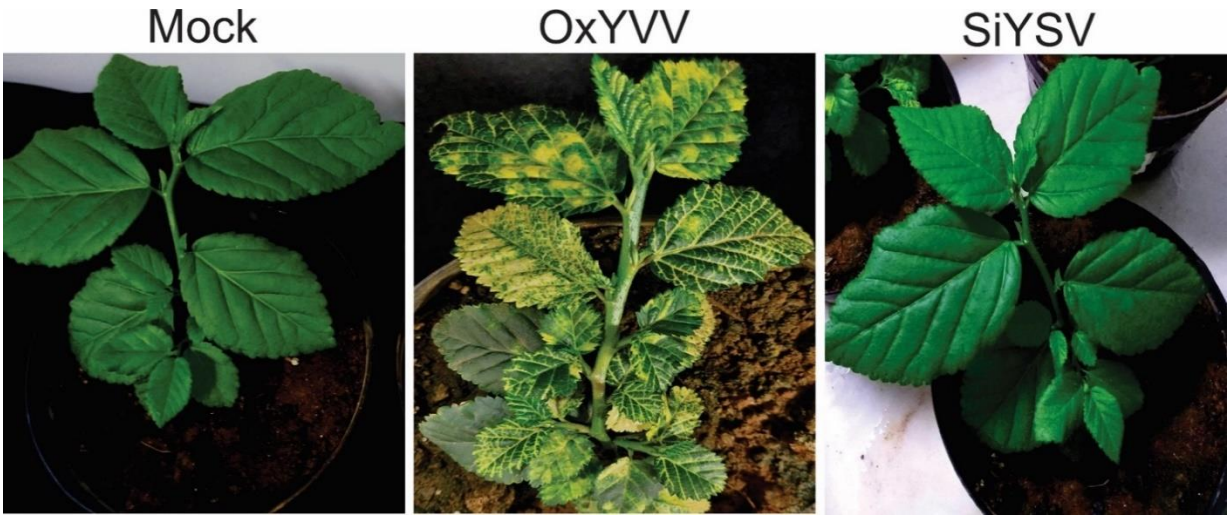


Figure 4

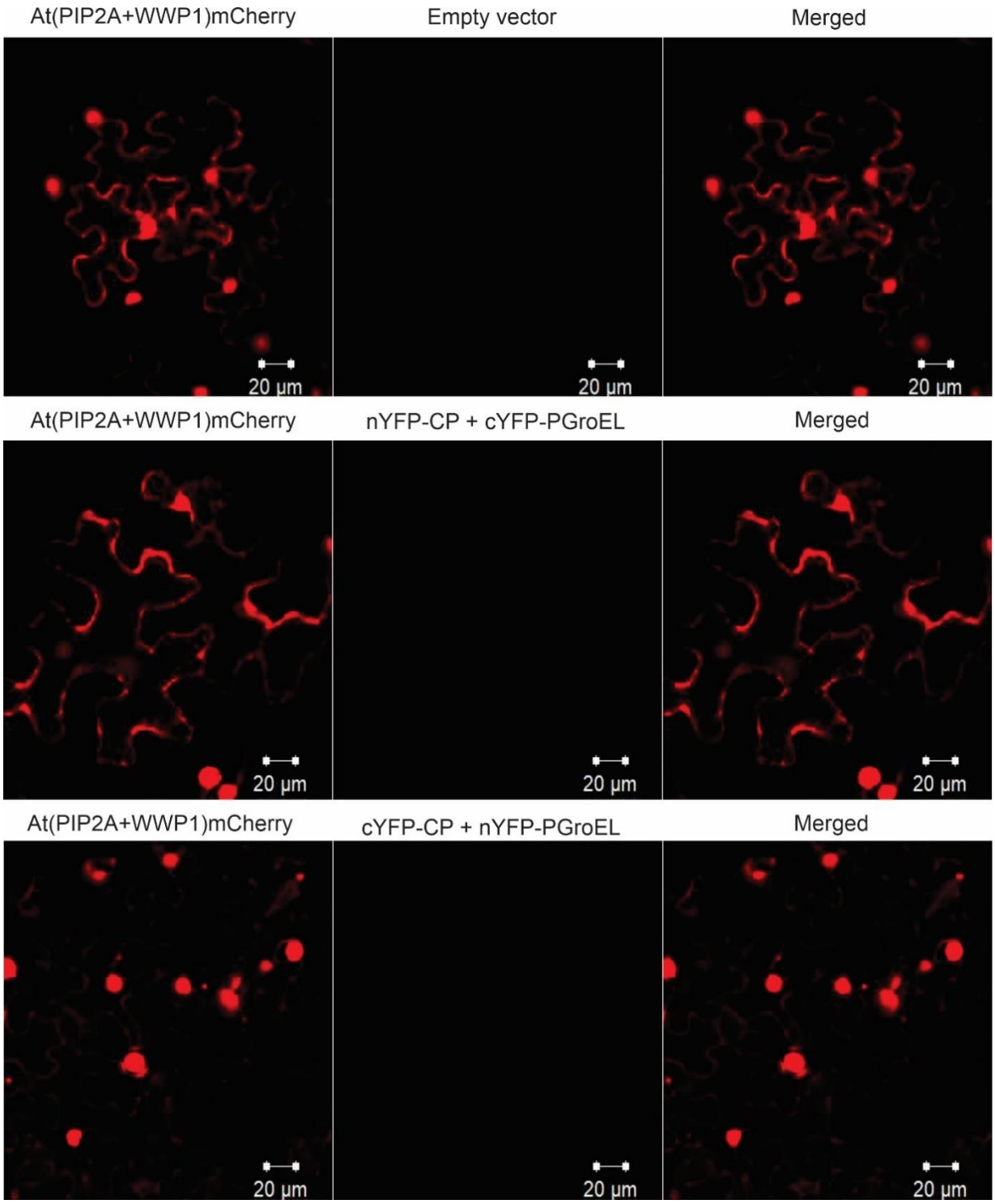

At(PIP2A+WWP1)mCherry
Empty vector
Merged
20 µm
20 µm
20 µm
At(PIP2A+WWP1)mCherry
nYFP-CP + cYFP-PGroEL
Merged
20 µm
20 µm
20 µm
At(PIP2A+WWP1)mCherry
cYFP-CP + nYFP-PGroEL
Merged
20 µm
20 µm
20 µm

Figure 6 (cont.)

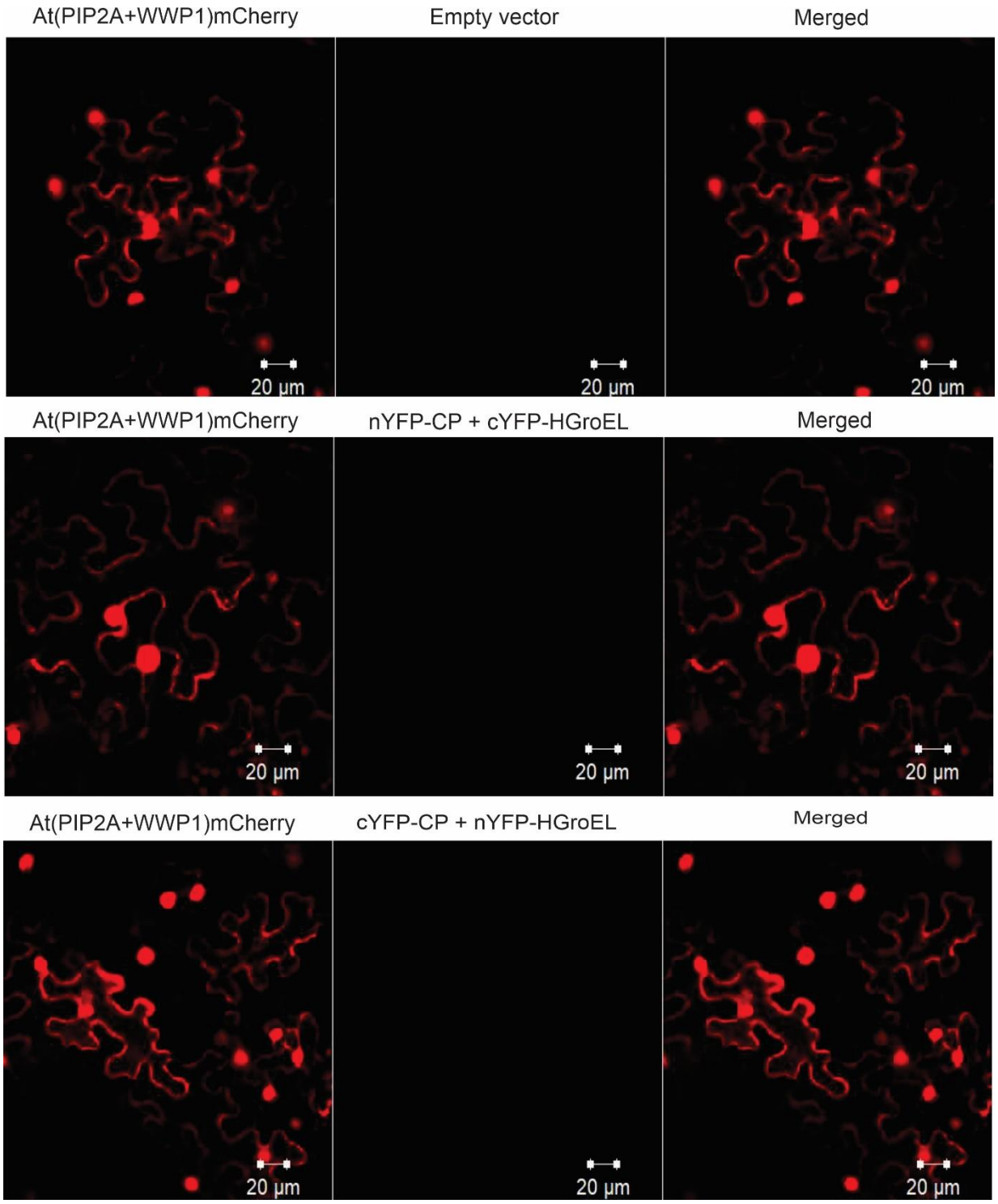


Figure 5

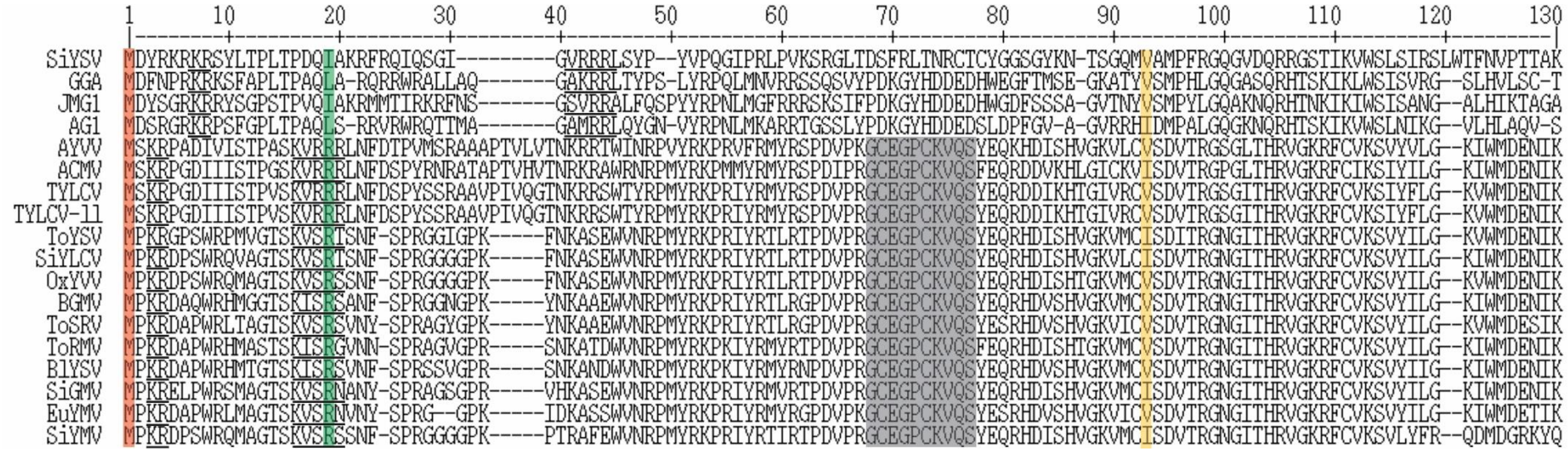

1 10 20 30 40 50 60 70 80 90 100 110 120 130
SiYSV MDYRKRKRSYLTPLTPDQIAKRFRQIQSGI---------GVRRRLSYP--YVPQGIPRLPVKSRGLTDSFRLTNRCTCYGGSGYKN-TSGQMVAMPFRGQGVDQRRGSTIKVWSLSIRSLWTFNVPTTAK
GGA MDFNPRKRKSFAPLTPAQLA-RQRRWRALLAQ--------GAKRRLTYPS-LYRPQLMNVRRSSQSVYPDKGYHDDEDHWEGFTMSE-GKATYVSMPHLGQGASQRHTSKIKLWSISVRG--SLHVLSC-T
JMG1 MDYSGRKRRYSGPSTPVQIAKRMMTIRKRFNS-------GSVRRALFQSPYYVRPNLMGFRRRSKSIFPDKGYHDDEDHWGDFSSSA-GVTNYVSMPYLGQAKNQRHTNKIKIWSISANG--ALHIKTAGA
AG1 MDSRGRKRPSFGPLTPAQLS-RRVRWRQTTMA-------GAMRRLQYGN-VYRPNLMKARRTGSSLYPDKGYHDDEDSLDPFGV-A-GVRRHIDMPALGQGKNQRHTSKIKVWSLNIKG--VLHLAQV-S
AYVV MSKRPADIVISTPASKVRRRLNFDTPVMSRAAAPTVLVTNKRRTWINRPVYRKPRVFRMYRSPDVPKGCEGPCKVQSYEQKHDISHVGKVLCVSDVTRGSGLTHRVGKRFCVKSVYVLG--KIWMDENIK
ACMV MSKRPGDIIISTPGSKVRRRLNFDSPYRNRATAPTVHVTNRKRAWRNRPMYRKPMMYRMYRSPDIPRGCEGPCKVQSFEQRDDVKHLGICKVISDVTRGPGLTHRVGKRFCIKSIYILG--KIWMDENIK
TYLCV MSKRPGDIIISTPVSKVRRRLNFDSPYSSRAAVPIVQGTNKRRSWTYRPMYRKPRIYRMYRSPDVPRGCEGPCKVQSYEQRDDIKHTGIVRCVSDVTRGSGITHRVGKRFCVKSIYFLG--KVWMDENIK
TYLCV-ll MSKRPGDIIISTPVSKVRRRLNFDSPYSSRAAVPIVQGTNKRRSWTYRPMYRKPRIYRMYRSPDVPRGCEGPCKVQSYEQRDDIKHTGIVRCVSDVTRGSGITHRVGKRFCVKSIYFLG--KVWMDENIK
ToYSV MPKRGPSWRPMVGTSKVSRTSNF-SPRGGIGPK-----FNKASEWVNRPMYRKPRIYRTLRTPDVPRGCEGPCKVQSYEQRHDISHVGKVMCISDITRGNGITHRVGKRFCVKSVYILG--KVWMDENIK
SiYLCV MPKRDPSWRQVAGTSKVSRTSNF-SPRGGGGPK-----FNKASEWVNRPMYRKPRIYRTLRTPDVPRGCEGPCKVQSYEQRHDISHVGKVLCISDVTRGNGITHRVGKRFCVKSVYILG--KIWMDENIK
OxYVV MPKRDPSWRQMAGTSKVSRSSNF-SPRGGGGPK-----FNKASEWVNRPMYRKPRIYRTLRTPDVPRGCEGPCKVQSYEQRHDISHTGKVMCVSDVTRGNGITHRVGKRFCVKSVYILG--KVWMDENIK
BGMV MPKRDAQWRHMGGTSKISRSANF-SPRGGNGPK-----YNKAAEWVNRPMYRKPRIYRTLRGPDVPRGCEGPCKVQSYEQRHDVSHVGKVMCVSDVTRGNGITHRVGKRFCVKSVYILG--KIWMDENIK
ToSRV MPKRDAPWRLTAGTSKVSRSVNY-SPRAGYGPK-----YNKAAEWVNRPMYRKPRIYRTLRGPDVPRGCEGPCKVQSYESRHDVSHVGKVICVSDVTRGNGITHRVGKRFCVKSVYILG--KVWMDESIK
ToRMV MPKRDAPWRHMASTSKISRGVNN-SPRAGVGPR-----SNKATDWVNRPMYRKPKIYRMYRTPDVPRGCEGPCKVQSFEQRHDISHTGKVMCVSDVTRGNGITHRVGKRFCVKSVYILG--KIWMDENIK
BlYSV MPKRDAPWRHMTGTSKISRSVNF-SPRSSVGPR-----SNKANDWVNRPMYRKPKIYRMYRNPDVPRGCEGPCKVQSYEQRHDVSHVGKVMCVSDVTRGNGITHRVGKRFCVKSVYIIG--KIWMDENIK
SiGMV MPKRELPWRSMAGTSKVSRNANY-SPRAGSGPR-----VHKASEWVNRPMYRKPRIYRMVRTPDVPRGCEGPCKVQSYEQRHDISHVGKVMCISDVTRGNGITHRVGKRFCVKSVYILG--KIWMDENIK
EuYMV MPKRDAPWRLMAGTSKVSRNVNY-SPRG--GPK-----IDKASSWVNRPMYRKPRIYRMYRGPDVPKGCEGPCKVQSYESRHDVSHVGKVICVSDVTRGNGITHRVGKRFCVKSVYILG--KIWMDETIK
SiYMV MPKRDPSWRQMAGTSKVSRSSNF-SPRGGGGPK-----PTRAFEWVNRPMYRKPRIYRTIRTPDVPRGCEGPCKVQSYEQRHDISHVGKVMCISDVTRGNGITHRVGKRFCVKSVLYFR--QDMDGRKYQ

Figure 6

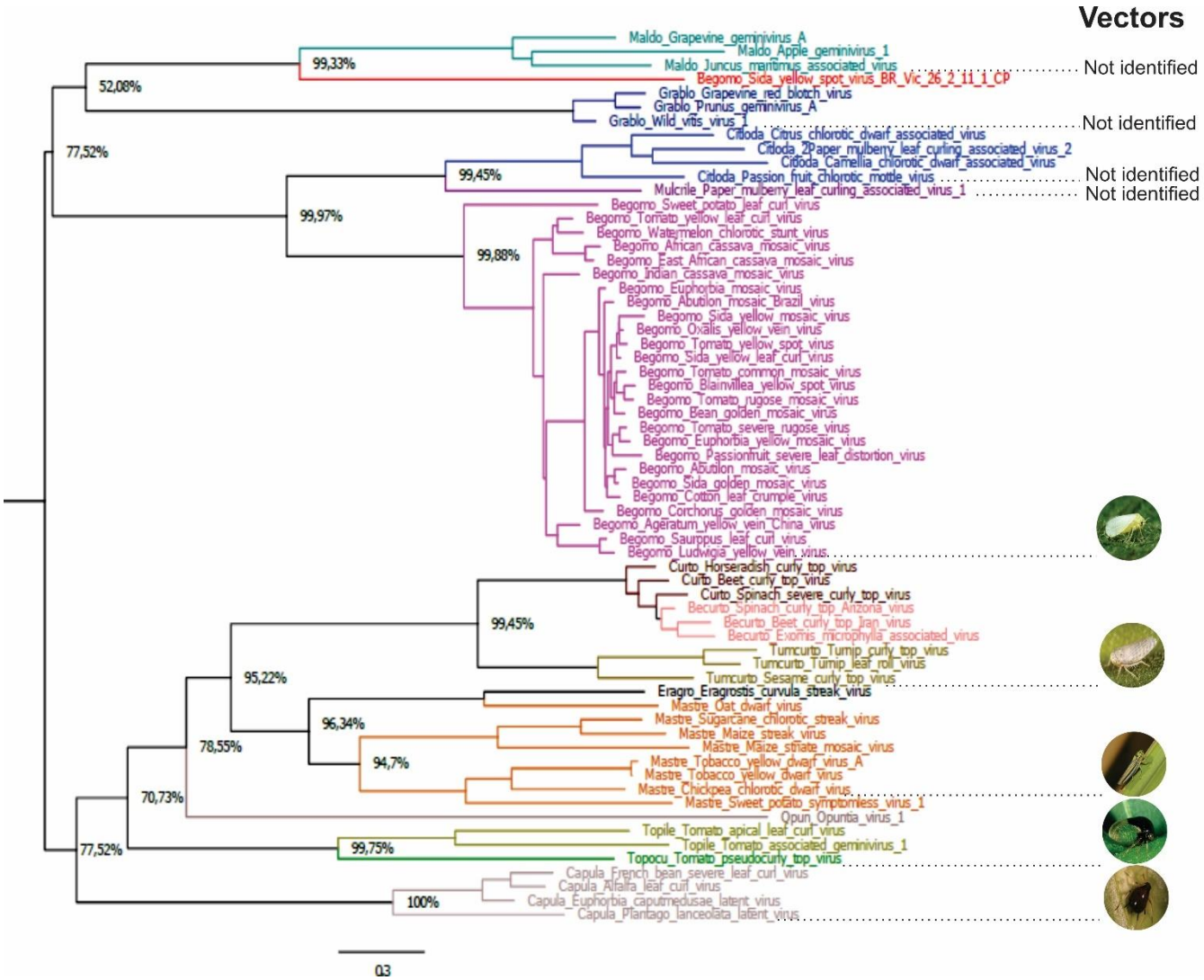

Vectors
Maldo_Grapevine_geminivirus_A
Maldo_Apple_geminivirus_1
Maldo_Juncus_maritimus_associated_virus
Begomo_Sida_yellow_spot_virus_BR_Vic_26_2_11_1_CP
Grablo_Grapevine_red_blotch_virus
Grablo_Prunus_geminivirus_A
Grablo_Wild_vitis_virus_1
Citloda_Citrus_chlorotic_dwarf_associated_virus
Citloda_2Paper_mulberry_leaf_curling_associated_virus_2
Citloda_Camellia_chlorotic_dwarf_associated_virus
Citloda_Passion_fruit_chlorotic_mottle_virus
Mulcrile_Paper_mulberry_leaf_curling_associated_virus_1
Not identified
Not identified
Not identified
Not identified
Begomo_Sweet_potato_leaf_curl_virus
Begomo_Tomato_yellow_leaf_curl_virus
Begomo_Watermelon_chlorotic_stunt_virus
Begomo_African_cassava_mosaic_virus
Begomo_East_African_cassava_mosaic_virus
Begomo_Indian_cassava_mosaic_virus
Begomo_Euphorbia_mosaic_virus
Begomo_Abutilon_mosaic_Brazil_virus
Begomo_Sida_yellow_mosaic_virus
Begomo_Oxalis_yellow_vein_virus
Begomo_Tomato_yellow_spot_virus
Begomo_Sida_yellow_leaf_curl_virus
Begomo_Tomato_common_mosaic_virus
Begomo_Blainvillea_yellow_spot_virus
Begomo_Tomato_rugose_mosaic_virus
Begomo_Bean_golden_mosaic_virus
Begomo_Tomato_severe_rugose_virus
Begomo_Euphorbia_yellow_mosaic_virus
Begomo_Passionfruit_severe_leaf_distortion_virus
Begomo_Abutilon_mosaic_virus
Begomo_Sida_golden_mosaic_virus
Begomo_Cotton_leaf_crumple_virus
Begomo_Corchorus_golden_mosaic_virus
Begomo_Ageratum_yellow_vein_China_virus
Begomo_Saurpus_leaf_curl_virus
Begomo_Ludwigia_yellow_vein_virus
Curto_Horseradish_curly_top_virus
Curto_Beet_curly_top_virus
Curto_Spinach_severe_curly_top_virus
Becurto_Spinach_curly_top_Arizona_virus
Becurto_Beet_curly_top_Iran_virus
Becurto_Exomis_microphylla_associated_virus
Turncurto_Turnip_curly_top_virus
Turncurto_Turnip_leaf_roll_virus
Turncurto_Sesame_curly_top_virus
Eragro_Eragrostis_curvula_streak_virus
Mastre_Oat_dwarf_virus
Mastre_Sugarcane_chlorotic_streak_virus
Mastre_Maize_streak_virus
Mastre_Maize_striate_mosaic_virus
Mastre_Tobacco_yellow_dwarf_virus_A
Mastre_Tobacco_yellow_dwarf_virus
Mastre_Chickpea_chlorotic_dwarf_virus
Mastre_Sweet_potato_symptomless_virus_1
Opun_Opuntia_virus_1
Topile_Tomato_apical_leaf_curl_virus
Topile_Tomato_associated_geminivirus_1
Topocu_Tomato_pseudocurly_top_virus
Capula_French_bean_severe_leaf_curl_virus
Capula_Alfalfa_leaf_curl_virus
Capula_Euphorbia_caputmedusae_latent_virus
Capula_Plantago_lanceolata_latent_virus
99,33%
52,08%
77,52%
99,45%
99,97%
99,88%
99,45%
95,22%
96,34%
78,55%
94,7%
70,73%
77,52%
99,75%
100%
0.3

Figure 7

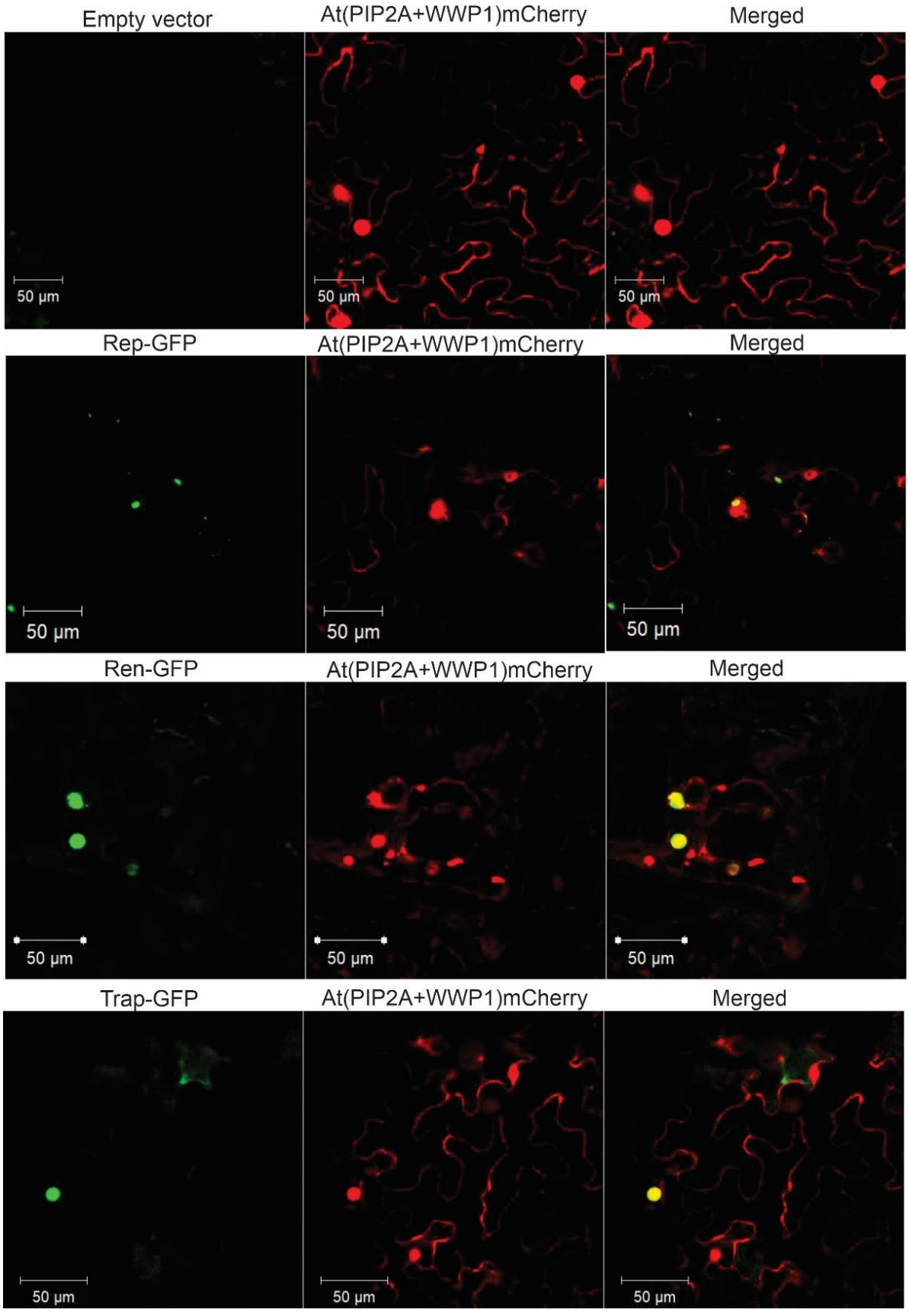

Empty vector
At(PIP2A+WWP1)mCherry
Merged
50 µm
50 µm
50 µm
Rep-GFP
At(PIP2A+WWP1)mCherry
Merged
50 µm
50 µm
50 µm
Ren-GFP
At(PIP2A+WWP1)mCherry
Merged
50 µm
50 µm
50 µm
Trap-GFP
At(PIP2A+WWP1)mCherry
Merged
50 µm
50 µm
50 µm

Figure 7 (cont.)

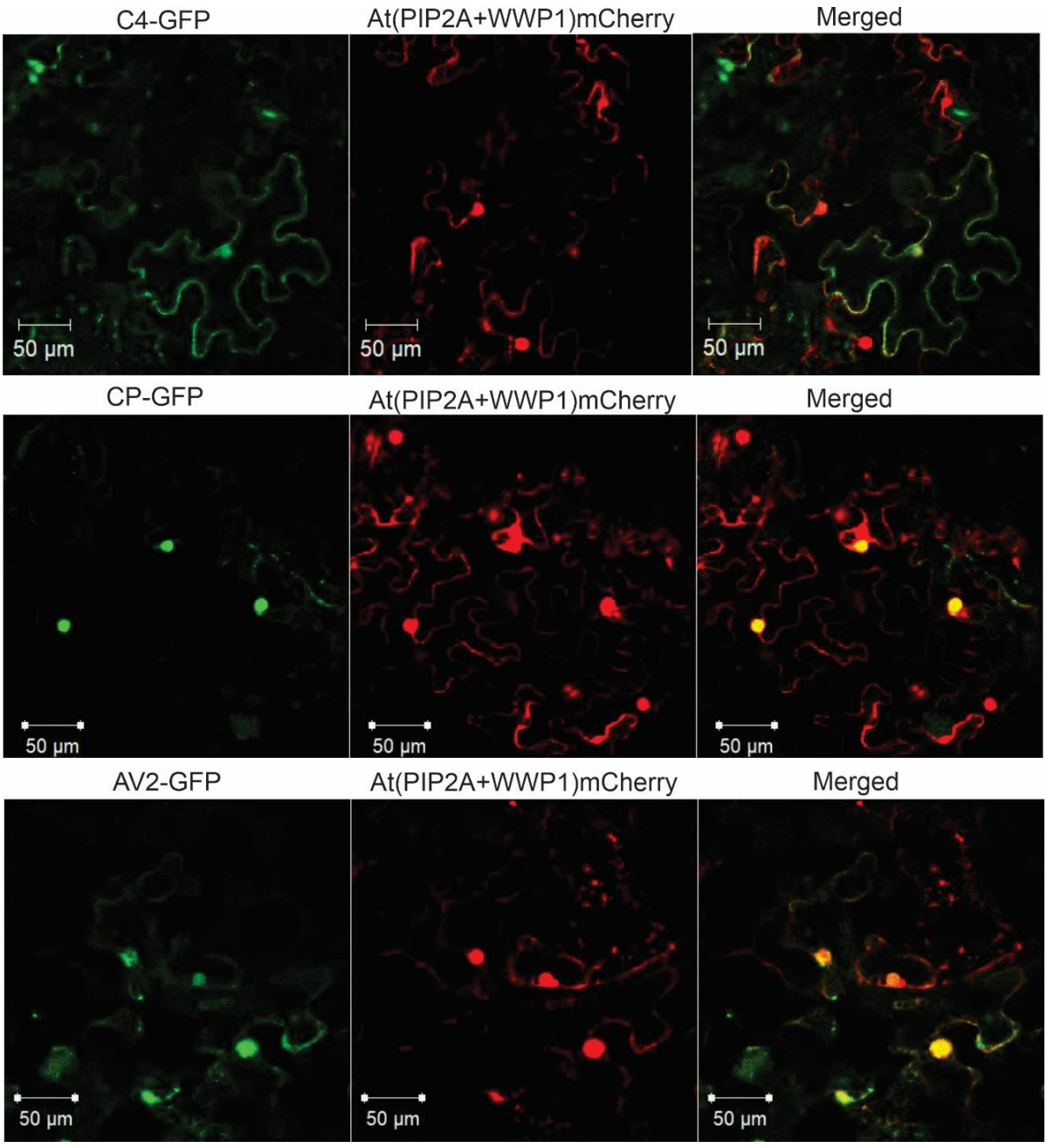
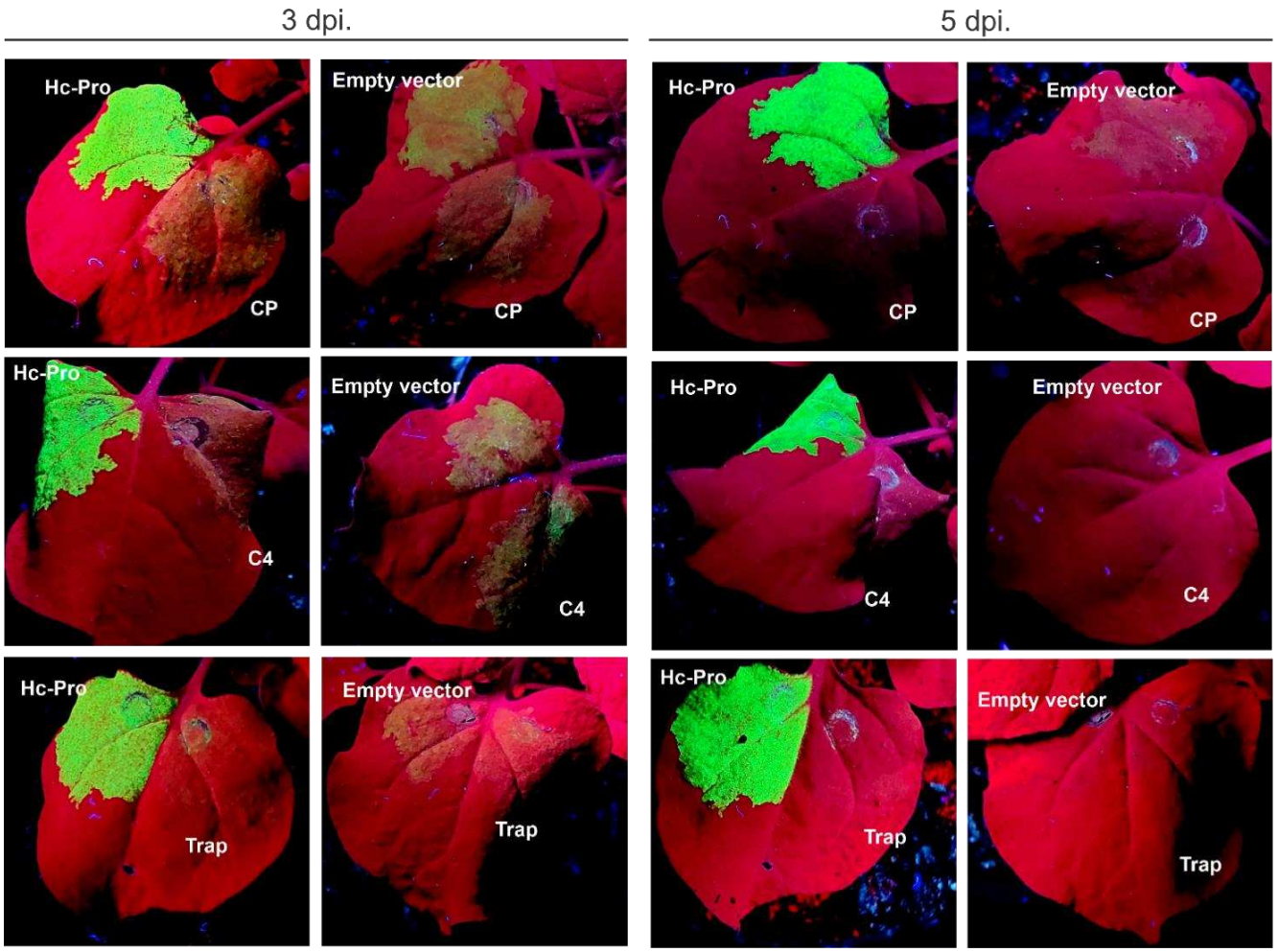


Figure 8



GENERAL CONCLUSIONS

- The preponderance of evidence indicates that SiYSV forms a typical geminivirus capsid.
- The lack of particle visualization by TEM could be due to low viral concentration in the plant tissues or at the time of sampling, or the capsid may be unstable and thus could have been disassembled during the dehydration/fixation procedure.
- SiYSV can infect other hosts, including a cultivated plant (okra), and thus could be a threat to economically important crops.
- The inability of *B. tabaci* MEAM1 to transmit SiYSV is probably related to the lack of interaction between CP and GroEL.
- It remains to be determined if other (indigenous) species in the *B. tabaci* complex can transmit SiYSV, or if the virus is transmitted by a different type of vector.